Geospatial and genomic tools for conserving the critically endangered blue-eyed black lemur (*Eulemur flavifrons*) and the sportive lemurs (genus *Lepilemur*)

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Abstract

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Madagascar's lemurs are the most endangered group of mammals in the world, with 94% of species threatened with extinction. Forest loss is one the greatest threat to these arboreal primates, but hunting, habitat degradation, and climate change also threaten their survival. Lemurs are a diverse group of more than 100 species; and their ecological traits shape how species respond to anthropogenic pressure. Incorporating knowledge of species' ecological niches and evolutionary histories can contextualize threats and improve conservation assessments. In this dissertation, I investigate what constitutes suitable habitat for lemurs in light of the threats present, their sensitivity to forest fragmentation, their dispersal ability, and their ecological uniqueness.

I obtained data about lemur distributions in two ways. First, I conducted field surveys of the Critically Endangered blue-eyed black lemur (*Eulemur flavifrons*), which only occurs in the ecotone between eastern rainforest and western dry forest in the Sahamalaza region. I also surveyed the range of sister species, the black lemur (*E. macaco*), which inhabits nearby eastern rainforest in the Manogarivo region. I focused on areas that have not been surveyed recently and on the poorly studied boundary between the species to collect observations from the breadth of these species' ecological ranges. I also documented threats, including incursions into protected areas, and collected fecal samples to test whether whole genomes could be obtained noninvasively for analyses of local adaptation in these species.

Second, I searched online databases and published literature for GPS localities for all species of lemur. I used these records, along with the ones collected in the field, to construct ecological niche models for nearly all species of lemur using Maxent. For the blue-eyed black lemur and the black lemur, I estimated the remaining area they can occupy based on these models and the threat survey data. Next, I examined the role of not just forest loss, but forest degradation, in determining where lemur species occur. I used high-resolution forest cover maps to determine lemurs' tolerance for characteristics of degraded forest, including distance to the edge and mean patch size. I then limited species niche model to only intact, forested habitat. Lastly, using the sportive lemurs (genus *Lepilemur*) as an example, I evaluate how the inability to disperse across large rivers has influenced ecological niche diversity. I also examine what limited dispersal ability will mean for these species as climate change causes their ranges to shift.

Field surveys in the Sahamalaza and Manongarivo regions revealed extensive threats to blue-eyed black lemurs, from traps to cattle incursions and fire. I found no evidence of sympatry, but did locate an undocumented population of *E. flavifrons* north of the Andranomalaza River. Madagascar National Parks (MNP) managed protected areas appear to have less human incursion than NGO-managed protected areas. Further investigation of the ecological distinctiveness of these species is possible via non-invasive methods: I sequenced whole genomes at 2.3x coverage from eight of the fecal samples collected during this study. While SNPs indicating a loss of function did not reveal any patterns, sequencing additional samples could make studies of local adaptation and population genetic diversity possible.

At the regional scale, forest conversion is a grave threat to lemurs. When forest loss and degradation are considered in habitat models, lemur species have lost 51% of their habitat in the last 30 years. Proximity to a forest edge rendered more forested areas too degraded for lemurs than did mean patch size. This result is likely the influence of human contact nearer the forest edge. I recommend urgent support for reserves like Beanka, Tsimembo Forest, Ranobe PK 32, and Amoron'i Onilahy, which have highly suitable, intact forest for many lemur species. Spaces like these will be important for conserving the remarkable diversity within the sportive lemur clade. Though their distribution is largely explained by riverine barriers, I show a role for ecological niche divergence and local adaptation in accelerating allopatric speciation. These same rivers will limit their ability to track climatically suitable areas as climate change progresses: sportive lemurs as a group will lose nearly a quarter of their accessible habitat to climate change by the 2070s.

While my results are focused on the particulars of lemur conservation in Madagascar, the methods I have presented here are broadly applicable to other threatened species. Piggybacking fecal sample collection onto rapid field surveys is straightforward. The possibility of obtaining whole genomes from non-invasive samples presents a new way to answer questions about local adaptation without risking injury to other arboreal study subjects, like Neotropical monkeys, or for elusive species like big cats. For threatened species, their climatic niche only dictates part of their distribution. The habitat quantification pipeline presented here takes advantage of thirty-five years of research in Madagascar to estimate species' tolerance for forest fragmentation. While these records are impressive for primates, they are dwarfed by those available for passerines, through scientific literature and online repositories like eBird. By integrating field surveys, ecological niche modeling, and non-invasive genomics, we can begin to understand the complex threats facing species like lemurs and the options for ensuring their survival.

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Dedication

To my hilarious, resilient mom, Edie Tinsman, whose joy in helping furry critters is infectious. She and Henry gave me every opportunity to find happiness.

To Ömer İkizler, who did everything from proofreading my grad school applications to making a home for us while I wrote this document. He has been my family and my friend the last ten years.

Chapter 1: Range and conservation updates for the Critically Endangered blue-eyed black lemur *Eulemur flavifrons* and the Vulnerable black lemur *Eulemur macaco*

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Abstract

The Critically Endangered blue-eyed black lemur *Eulemur flavifrons* of north-western Madagascar is one of the most threatened primates. The majority of research and conservation efforts for the species have been restricted to the Sahamalaza Peninsula but there are unstudied and unprotected populations farther inland. The dearth of information regarding the transition between *E. flavifrons* and its parapatric sister species, the Vulnerable black lemur *Eulemur macaco*, and the possibility of a hybrid population complicates conservation planning for both species. We surveyed 29 forest fragments across both species' ranges to investigate the boundary between the taxa, whether hybrids persist, and the threats to lemurs in the region. We found *E. flavifrons* in six fragments and *E. macaco* in seventeen. We never observed *E. flavifrons* and *E. macaco* in the same location and we found no conclusive evidence of hybrids. Three fragments in which *E. flavifrons* was present were north of the Andranomalaza River, which had been previously considered the barrier between the two species. Based on these observations and a literature review, we provide updated ranges, increasing the Extent of Occurrence (EOO) of *E. flavifrons* by 28.7% and reducing the EOO of *E. macaco* by 44.5%. We also evaluate the capacity of protected areas to conserve these lemurs. We recommend additional surveys and the implementation of an education programme in this region to help conserve both species.

Keywords

Black lemur, blue-eyed black lemur, geographical range, Madagascar, Manongarivo Special Reserve, Sahamalaza–Iles Radama National Park, species distribution

Introduction

Madagascar, a biodiversity hotspot (Myers et al., 2000), is home to > 100 endemic species of lemurs, accounting for > 20% of global primate diversity; however, 94% of lemur species are threatened by hunting and deforestation (Schwitzer et al., 2014). One of the species most affected is the Critically Endangered blue-eyed black lemur *Eulemur flavifrons*, which is subject to poaching and habitat loss as a result of slash-and-burn rice cultivation (*tavy*), logging and livestock rearing (Andrianjakarivelo, 2004; Seiler et al., 2010; Andriaholinirina et al., 2014a). The population of blue-eyed black lemurs declined by > 80% during 1990–2014 (Andriaholinirina et al., 2014a).

Estimates of *E. flavifrons* numbers have focused mainly on the protected population in the Ankarafa Forest of Sahamalaza–Iles Radama National Park, where there are estimated to be 60–130 individuals/km^2 (Schwitzer et al., 2006; Volampeno et al., 2010). Surveys of the isolated fragments where *E. flavifrons* occurs farther inland found much lower densities, with a mean of 24 individuals/km^2 (Andrianjakarivelo, 2004). Based on these surveys there are only an estimated 2,780–6,950 individuals remaining (Schwitzer et al., 2006). A population viability analysis concluded that the Ankarafa population, which is the largest remaining, could be extirpated by 2026 (Volampeno et al., 2015). The vulnerability of *E. flavifrons* is partially attributable to its unique habitat; the species occurs only in the transitional, subtropical forest between Madagascar's western dry deciduous forests and the humid evergreen rainforests of the east (Schwitzer et al., 2007). The plant, amphibian, reptile and mammal communities of the Sahamalaza Peninsula include endemic species that occur nowhere else on the island (Birkinshaw, 2004; Schwitzer et al., 2006; Penny et al., 2017).

Although not confined to the Sahamalaza Peninsula, *E. flavifrons* has one of the smallest geographical ranges of the genus *Eulemur* (Volampeno et al., 2010). Its estimated Extent of Occurrence (EOO; i.e. the smallest, continuous area encompassing all known or projected occurrences of a species; IUCN, 2001) is < 2,700 km^2 (Andriaholinirina et al., 2014a). Its Area of Occupancy (AOO; i.e. the area of suitable habitat that is actually occupied within a species' EOO; IUCN, 2001) must be even smaller, although it had not been estimated previously.

The EOO of *E. flavifrons* is bounded by the Mozambique Channel to the west and the Maevarano River to the south (Koenders et al., 1985; Petter and Andriatsarafara, 1987; Randriatahina and Rabarivola, 2004; Schwitzer and Lork, 2004; Andriaholinirina et al., 2014a; Fig. 1.1). It extends east to the Sandrakota River and Manongarivo Special Reserve, which comprises 32,000 ha of protected Sambirano rainforest (MEF and MNP, 2010). Previous studies have identified the Andranomalaza River, also called the Maitsomalaza in the local Sakalava dialect, as the boundary between *E. flavifrons* and its parapatric sister species, the black lemur *Eulemur macaco* (Koenders et al., 1985; Andriaholinirina et al., 2014a).

However, there is conflicting evidence regarding whether the lemurs between the Andranomalaza River and the more northern Manongarivo River are hybrids, intermediate-appearing forms on a phenotypic cline, or typical members of either species (Meyers et al., 1989; Rabarivola et al., 1991; Andrianjakarivelo, 2004; Randriatahina and Rabarivola, 2004).
The first report of phenotypic variation was by Meyers et al. (1989), who observed two distinct groups of lemurs unlike typical *E. flavifrons* or *E. macaco*. The first group was at Beraty, and

individuals had light brown eyes and short ruffs of hair around their ears, characteristics that are intermediate between *E. flavifrons* and *E. macaco*. The second group was at Ambodivoahangy, and individuals had darker eyes and redder coats than is typical of Sahamalaza *E. flavifrons*. However, when Andrianjakarivelo (2004) visited Ambodivoahangy he found animals that 'greatly resembled' *E. flavifrons*. Goodman and Schütz (2000) surveyed the eastern slopes of Manongarivo Special Reserve, north of Ambodivoahangy, and identified groups containing both *E. macaco* and 'hybrid' individuals but did not detail their criteria for these distinctions. Their assessment was complicated by the presence of *E. fulvus* in that area, which may be perceived as having a reddish coat (Goodman and Schütz, 2000).

Updated assessments of the lemurs in this region are necessary to establish effective conservation initiatives for these two species (Rakotonirina et al., 2011, 2014). They could also improve estimates of the species' ranges, especially considering the area has not been assessed since 2004 (Schwitzer et al., 2014). We investigated the presence and phenotypes of *Eulemur* species from Sahamalaza–Iles Radama National Park to Manongarivo Special Reserve, and the threats to their survival, to (1) establish the continued existence of *E. flavifrons* outside protected habitat, (2) locate the purported contact zone between *E. flavifrons* and *E. macaco*, and (3) understand anthropogenic pressures in and around the protected areas in this region. We provide updated EOOs and new AOOs for both species, and report on the status of the lemurs between the Manongarivo and Andranomalaza Rivers.

Methods

We conducted surveys during June-September and November-December 2015, June 2016 and April-June 2017. To evaluate possible barriers between *E. flavifrons* and *E. macaco* we worked eastwards from the coast near Maromandia (-14.17467, 48.12539) to the north-eastern slopes of Manongarivo Special Reserve (-14.01189, 48.37981), focusing our efforts around the Andranomalaza and Manongarivo rivers. We visited eight sites within and 10 outside the Reserve (Fig. 1.2) and 11 other sites throughout the region to record threats to lemur survival and to observe typical members of both species (Figs. 1.2, 1.3). We searched for lemurs for up to 7 days at each site, calling the site an absence if we could not find lemurs after a week of diurnal surveys led by a local person who worked in the forest. We walked the interior of each fragment, relying on paths when possible, until we heard lemur vocalizations or movement. We followed the lemurs until they would settle down to sleep, enabling their observation. We recorded global positioning system (GPS) coordinates for all observed *Eulemur* spp. and noted key morphological features (eye color, presence of ruffs) to distinguish between *E. flavifrons* and *E. macaco*. Evidence of *tavy*, livestock incursions, traps, and hunting was also noted.

In addition to field surveys we conducted a thorough review of the literature for occurrence records of *E. flavifrons* and *E. macaco*. We searched four online databases (ReBioMa, Manis, VertNet and GBIF) for *Eulemur* spp. records. All relevant articles published in *Lemur News*, *Madagascar Conservation and Development*, *Primate Conservation* and *Malagasy Nature* were scanned visually for GPS coordinates. We also conducted several searches in Google Scholar using combinations of the following terms: *Eulemur macaco*, *Eulemur flavifrons*, GPS, occurrence, coordinates, and range. These articles, databases and our field efforts yielded 119 unique records for *E. flavifrons* and 182 for *E. macaco* (Supplementary Table 1.1). New EOOs were determined by comparing these records to the river catchments in this region. River data were downloaded from WWF's HydroSHEDS project (Lehner et al., 2008).

To approximate AOOs for these species we used these occurrence records to construct ecological niche models. Points were thinned to no closer than 2.5 km apart, to reduce spatial autocorrelation (Kramer-Schadt et al., 2013). Environmental data related to an arboreal life history were downloaded from WorldClim, CliMond, SoilGrids, WorldGrids, and CIRCAD (see Supplementary Table 1.2 for data types and sources). Only variables with relatively low correlation to each other ($|r| < 0.85$; mean $|r| = 0.38$) were included in analyses to reduce model overfitting (Dormann et al., 2013; Pearson et al., 2014). Niche models were constructed with Maxent 3.4.1 (Phillips et al., 2017) using parameters identified with ENMeval (Supplementary Table 1.3; Muscarella et al., 2014). A 10% training threshold was used to turn models into a binary prediction of 1 (suitable habitat) or 0 (unsuitable). The model for each species was then limited to its EOO and the most recent forest cover estimate available (Vieilledent et al., 2018). They were resampled to 2 km^2 resolution, which is the IUCN standard for AOO, before the area was calculated.

Results

We observed *E. flavifrons* in six of the 29 forest fragments surveyed (21% of sites; Table 1.1), and *E. macaco* in 17 fragments (59%). We observed *E. fulvus* at four sites, twice on its own and twice co-occurring with *E. macaco*. At four of the sites (14%) we found no individuals of any *Eulemur* species, and we never observed *E. flavifrons* and *E. macaco* in the same forest fragment. The lemurs we observed in previously unstudied areas near Antsahabilahy and along the Maherivaratra mountain range had pale eyes and no ruffs and appeared to be *E. flavifrons*, despite occurring north of the Andranomalaza River, within the IUCN-identified EOO for *E. macaco* (Plate 1, Fig. 1.2; Andriaholinirina et al., 2014b).

The only lemurs we observed that did not resemble typical members of either species were a few male lemurs at Beraty in Manongarivo Special Reserve (Plate 1). These males had shorter ruffs than other male *E. macaco* we had seen previously. Given that the females all resembled typical *E. macaco* and these individuals occurred at the southern end of their range, they could simply be clinal variants. Thus, we tentatively identify them as *E. macaco*. On the north-eastern slopes of the Reserve, at another possible hybrid site we visited, we saw only typical *E. macaco* and *E. fulvus*. We were unable to locate any lemurs at Ambodivoahangy, the last possible hybrid site, despite extensive searching.

Although we observed *E. flavifrons* north of the Andranomalaza River, we never observed the species north of the Manongarivo River, nor did we observe *E. macaco* south of the Manongarivo. We therefore propose that the Manongarivo and its tributary, the Antsahakolana River, form the boundary between the two species (Fig. 1.2). We generated a new EOO polygon for *E. flavifrons* based on these findings, increasing its EOO by 29%, from 2,700 to 3,475 km^2 (Andriaholinirina et al., 2014a; Fig. 1.2).

In addition to changing the southern boundary of *E. macaco* to the Manongarivo River, we concluded the species is bounded in the east by the Ifasy River, as our literature search revealed no records east of the river (Figs. 1.1, 1.2). In the south-east *E. macaco* was bounded by the Tsaratanana Reserve for similar reasons, although such limited surveys have been conducted in this region that this should be considered a low-confidence boundary. This revision reduces the EOO for *E. macaco* to 6,510 km^2, only 55% of the previous estimate of 11,740 km^2 (Andriaholinirina et al., 2014b). When these EOOs are limited to suitable habitat and remaining forest cover, *E. macaco* has an AOO of $\leq$ 1,992 km^2 and *E. flavifrons* of $\leq$ 884 km^2.

Throughout their ranges these lemurs endure extensive habitat disturbance and other anthropogenic threats. Subsistence hunting and tavy were present in a majority of the 29 surveyed locations (69%, n = 20; Table 1.1), including 100% of the *E. flavifrons* locations we visited. Only nine sites had no evidence of hunting or tavy. Eight were within protected areas managed by Madagascar National Parks and one was a heavily trafficked site on Nosy Komba where local people procure lemurs to entertain tourists. There was evidence of hunting, tavy or both in all four protected areas managed by an NGO, whereas there were threats present in only three of the 11 sites managed by Madagascar National Parks (Table 1.1).

Discussion

Although these changes in EOO do not warrant immediate adjustments to the species' IUCN Red List status, the range contraction for *E. macaco* is of concern and suggests the need for updated population estimates (Volampeno et al., 2010, 2015). A census of the newly identified population of *E. flavifrons* at Maherivaratra and Antsahabilahy is also needed urgently. The area it occupies south of Manongarivo Special Reserve is part of the largest continuous forest in this species' AOO (Fig. 1.3), and this population may be the largest remaining without any protection.

We were unable to confirm the ongoing presence of hybrid lemurs, despite visiting three of the four sites where they had been reported previously (Supplementary Table 1.1). Surveys between the headwaters of the Antsahakolana and Sandrakota Rivers are needed to determine a more accurate boundary between *E. flavifrons* and *E. macaco* and to collect genetic samples to address the question of any potential hybridization.

During our surveys we encountered traps, hunters, livestock, and/or *tavy* in 20 of the 29 study sites (Table 1.1), indicating that disturbance is an ongoing concern both inside and outside protected areas. The recent pressures on these lemur populations were described by our local guide for the Bemabaza fragment, where we observed fewer than a dozen *E. macaco* in 2016. He informed us that there had been nearly 100 lemurs 5 years previously but most of these had been wiped out by hunting. This increased pressure may be partly a result of cultural shifts. This region was traditionally home to the Sakalava people, for whom lemur consumption is taboo (Ramanantsoa, 1976; Harpet et al., 2000); however, there has been a recent influx of Tsimihety people (Wilson, 1971; Feeley-Harnik, 1980), who consume primates (Golden and Comaroff, 2015).

Given the level of habitat exploitation and hunting we observed in this region we suggest a multifaceted approach to conserving both species, as well as protecting the remaining forest fragments. In the near term additional surveys are needed to measure population numbers accurately (Salmona et al., 2014), evaluate habitat quality in these fragments (Irwin et al., 2005) and assess the impact the various threats reported here have had on *E. flavifrons* and *E. macaco* (Rakotonirina et al., 2011; Ravaloharimanitra et al., 2011).

In general, we observed fewer threats to lemurs in areas managed by Madagascar National Parks than in those managed by NGOs (Table 1.1); however, this dichotomy is confounded by a few factors. Well-protected sites within Manongarivo Special Reserve had one of two factors in their favour: frequent park staff presence or steep terrain unsuitable for rice cultivation or cattle ranching. All the NGO-managed protected areas we visited were closer to human settlements, arable, and unpatrolled by enforcement authorities.

The new-to-science population of *E. flavifrons* is in relatively accessible forest, but park patrols or on-site staff would help protect these animals. These options would be a possibility if the previously proposed expansion of Manongarivo Special Reserve happens (MEFT and MEM, 2008). This expansion would include the newly identified populations of *E. flavifrons* reported here and the largest block of suitable forest remaining for this species (Fig. 1.3).

However, increasing of the size of the Reserve will not achieve protection for these lemurs until the ongoing problems at its current borders are addressed (Gardner et al., 2018). The remote, low-lying areas in and south of the Reserve, including Ambodivoahangy and the proposed area of expansion, are experiencing ongoing forest loss; and the *tavy* and poaching we report here have been a problem for the Reserve since at least 2010 (MEF and MNP, 2010). These incursions are in part because of the lack of boundary markers around the Reserve and the difficulty in patrolling remote areas. Additional funding to address these concerns, coupled with increased researcher presence in this region, would help to deter deforestation (Seiler et al., 2010; Campera et al., 2017). Poaching is probably driven by the lack of readily available protein in this region; we saw children with kwashiorkor in several of the towns we visited south of the Reserve. In Madagascar, domestic meats are generally preferred to bushmeat. Therefore, providing chickens or fish stocks to these communities could reduce the poaching pressure on lemurs (Jenkins et al., 2011).

We also propose expanding the community-based conservation education initiative of the Association Européenne pour l'Etude et la Conservation des Lémuriens, which increases local support for conservation by teaching > 2,000 students on the Sahamalaza Peninsula about *E. flavifrons*, the Association's flagship species (Randriatahina, 2013). We suggest expanding the programme westwards to include the communities near the newly discovered population of *E.*

flavifrons, as well as incorporating aspects of community-based monitoring into the initiative. Community monitoring schemes have been established elsewhere in Madagascar to engage local people in forest management and the collection of species abundance and demographic data (Rakotonirina et al., 2011; Ravaloharimanitra et al., 2011). Such a programme would promote conservation (Ratsimbazafy, 2003), foster positive attitudes towards the environment (Balestri et al., 2017), and reduce hunting in and around the Reserve (Nadhurou et al., 2017).

Continued surveying of Critically Endangered species such as *E. flavifrons* is vital for establishing range distributions and identifying anthropogenic pressures on taxa living in increasingly fragmented forests. However, our research and future work should serve as a foundation for urgent, practical efforts to conserve these species. We hope that the identification of new EOOs and AOOs for two priority lemur species, and the threats facing these species, will help the Association Européenne pour l'Etude et la Conservation des Lémuriens and community stakeholders as they collaborate to protect the few remaining populations of *E. flavifrons* and *E. macaco*.

Table 1.1. Presence/absence of lemurs and threats to their survival recorded in surveys of forest fragments in Madagascar (Figs. 1.2, 1.3) conducted during 2015-2017.

Forest fragment		Year	Protected area*	Species	Traps/evidence of hunting	*Tavy*
1	Ambavanambahatra	2016	MNP	*E. fulvus*	-	-
2	Ambodimanga	2015		*E. flavifrons*	Yes	Yes
3	Ambodivoahangy	2015	MNP		Yes	Yes
4	Ambohitsara	2016	MNP	*E. fulvus*	-	-
5	Ampapanabe	2015			Yes	Yes
6	Analafady	2015		*E. macaco*	-	Yes
7	Andokobe	2015		*E. macaco*	Yes	Yes
8	Andranomatavy	2015	NGO	*E. macaco*	Yes	Yes
9	Angodrahely	2017		*E. flavifrons*	Yes	Yes
10	Ankazomena	2016	MNP	*E. macaco*	-	-
11	Antsahabilahy A	2015		*E. flavifrons*	Yes	Yes
12	Antsahabilahy B	2015			Yes	Yes
13	Befalafa	2015	MNP	*E. macaco*	-	-
14	Bekiritsana	2015	MNP	*E. macaco*	-	Yes
15	Bemabaza	2015		*E. macaco*	Yes	Yes
16	Beraty	2015	MNP	*E. macaco*, *E. fulvus*	-	-
17	Bevazimba	2016			-	Yes
18	Bongomirahavavy	2015	NGO	*E. macaco*	Yes	-
19	Galoko	2015	NGO	*E. macaco*	Yes	Yes
20	Kalobinono	2015	NGO	*E. macaco*	Yes	Yes
21	Kapany	2015	MNP	*E. macaco*	-	-
22	Lokobe	2015	MNP	*E. macaco*	-	-
23	Mahadera	2016		*E. macaco*	-	Yes
24	Maherivaratra A	2015		*E. flavifrons*	Yes	Yes
25	Maherivaratra B	2016		*E. flavifrons*	-	Yes
26	Mandriranabe	2015		*E. macaco*	Yes	Yes
27	Manongarivo[1]	2015	MNP	*E. macaco*, *E. fulvus*	-	-
28	Nosy Komba	2015		*E. macaco*	-	-
29	Sahamalaza (Ankarafa)	2015	MNP	*E. flavifrons*	Yes	Yes

*MNP, protected area managed by Madagascar National Parks; NGO, protected area managed by the Missouri Botanical Garden.

[1] *E. macaco* and *E. fulvus* were resting in the same tree together

Plate 1.1. Typical *Eulemur flavifrons* (left; (a) male and (d) female) and *E. macaco* (right; (c) male and (f) female), with a possible intermediate form from Beraty (center; (b) male), with similar eye color but a shorter ruff compared to *E. macaco*. (e) Putative *E. flavifrons* (female) from Antsahabilahy A (Table 1.1), with pale eyes and no ruff.

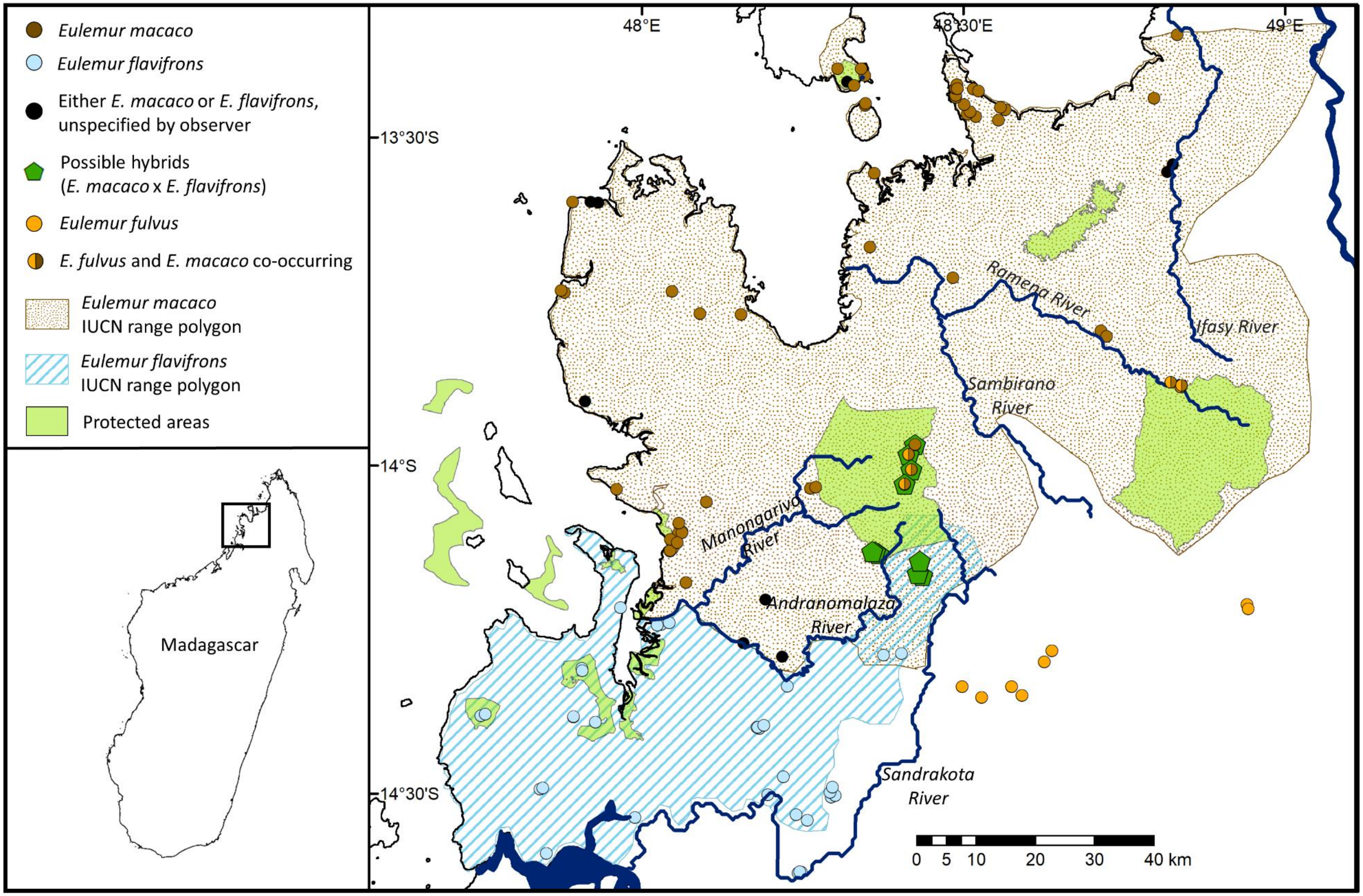


Figure 1.1. Previous Extent of Occurrence polygons for *Eulemur* sp., from IUCN and used with permission. All known occurrence records for *E. flavifrons*, *E. macaco* and putative hybrids, and for *E. fulvus*, in the area are noted.

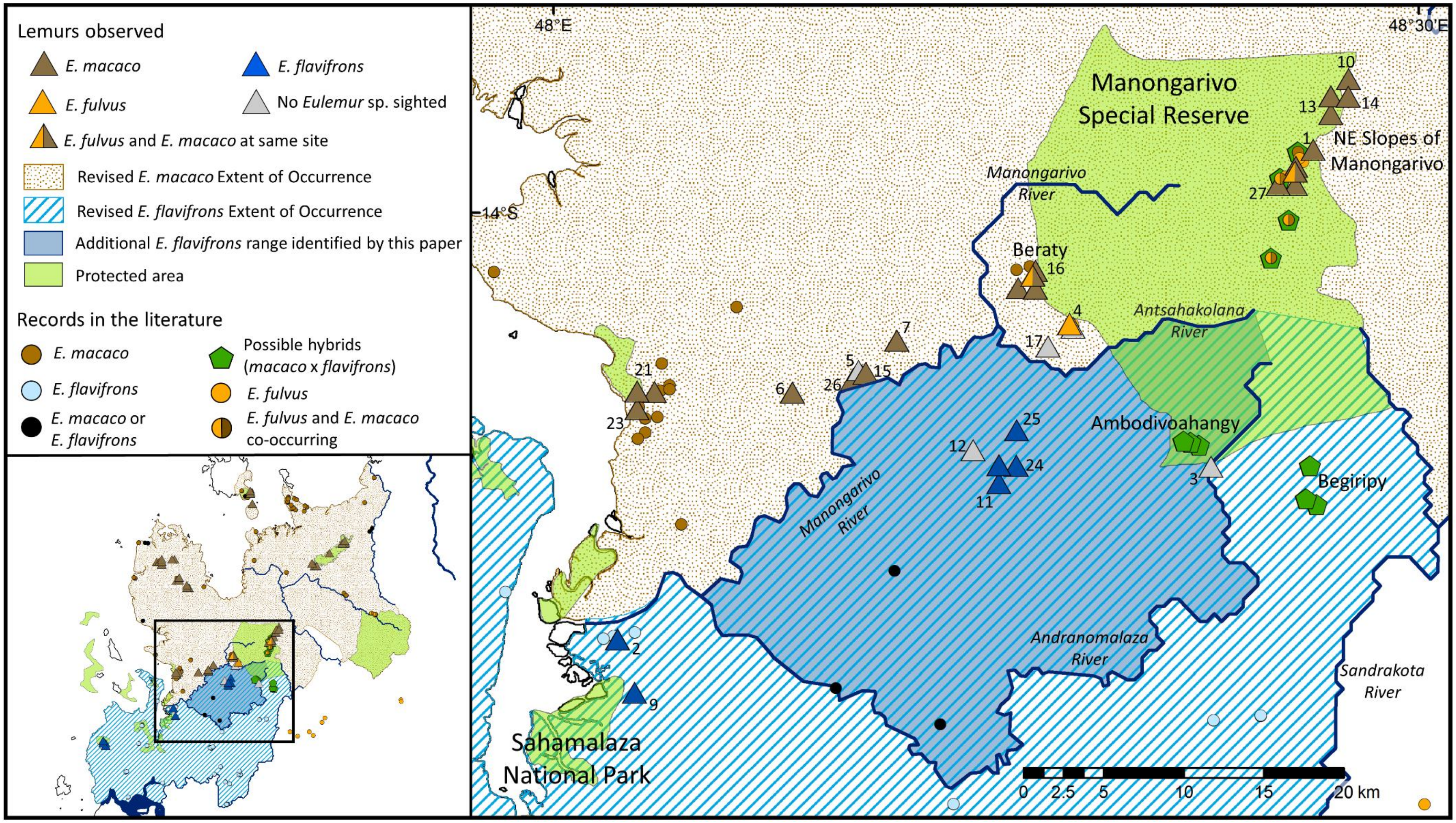


Figure 1.2. Updated Extent of Occurrence polygons for *Eulemur flavifrons* and *E. macaco* based on field work conducted in this study (triangles) and previous published studies (circles). Numbers correspond to forest fragments in Table 1.1.

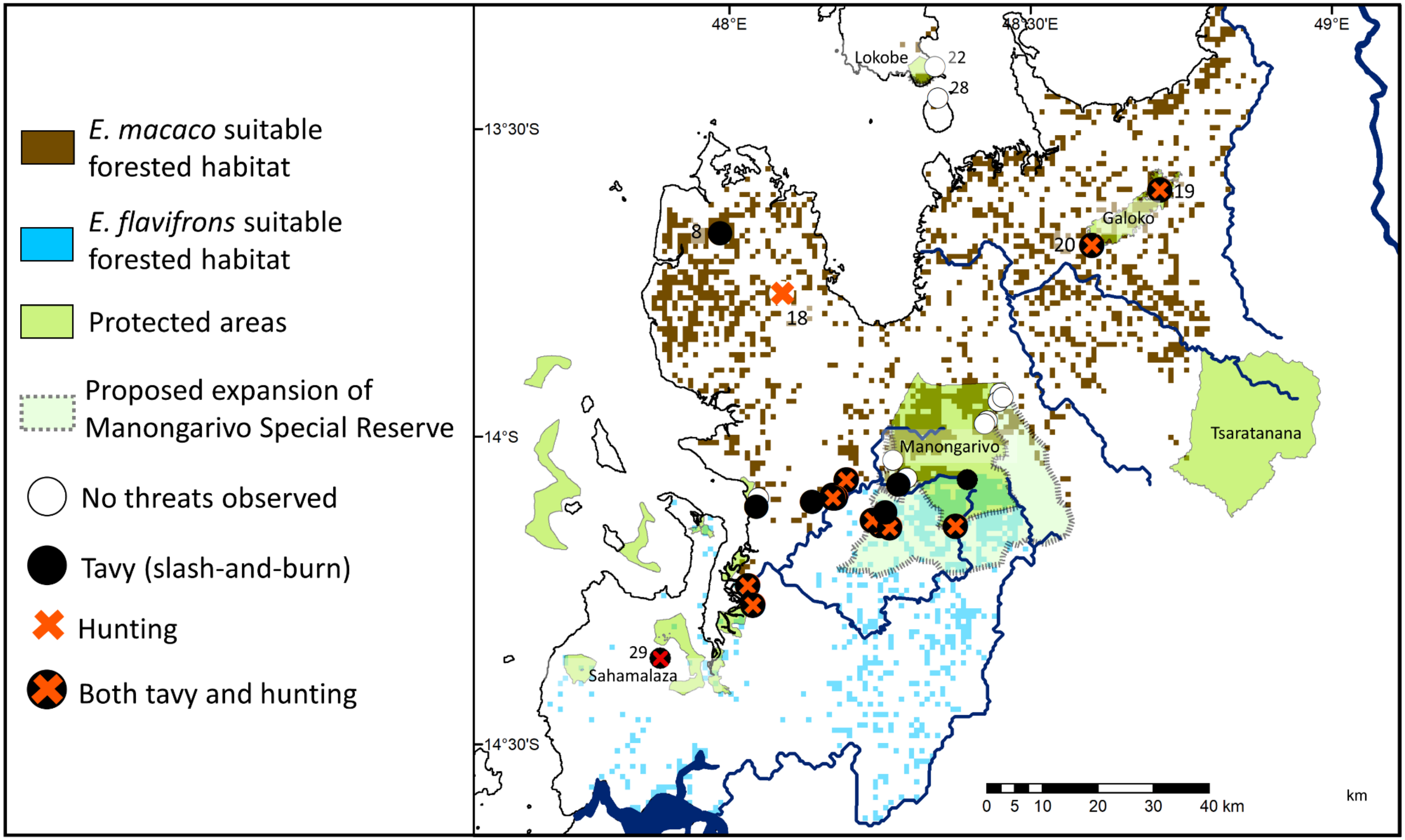


Figure 1.3. Extent of remaining suitable habitat for *E. flavifrons* and *E. macaco*, and threats observed throughout the study area. Numbers correspond to forest fragments listed in Table 1.1.

Whole genomes generated from non-invasively collected wild lemur samples

Abstract

The use of genomics in conservation has increased exponentially as next generation sequencing has become more readily available. Genomic data can provide more information about local adaptation, phylogenetic history, and population diversity than traditional genetics. However, the study of non-model organisms and remote wild populations still poses challenges. Minimally invasive samples like hair and feces have been a source of genetic data for these difficult-to-sample animals, but they have not traditionally yielded the amount of target species DNA required for massively parallel sequencing. Recent developments in DNA capture, which enriches for target species DNA, have finally put genomics within reach for minimally invasive samples. In this paper, I demonstrate an extension of a currently available DNA capture method, FecalSeq, by obtaining whole nuclear genomes from lemur fecal samples. These samples were collected from wild populations of two highly arboreal primates, the Critically Endangered blue-eyed black lemur (*Eulemur flavifrons*) and the Endangered black lemur (*E. macaco*), and stored in RNAlater at ambient temperature for up to six months. I sequenced whole lemur genomes from eight samples, with median coverage of 2.3x. These genomes yielded 41,374 reliable, high-quality SNPs. These results demonstrate the possibility of obtaining whole genomes from heavily contaminated, low target-DNA samples when invasive methods are not available.

Keywords

Minimally invasive sampling, fecal samples, whole genomes, DNA capture, blue-eyed black lemur, black lemur

Introduction

Capture is the most stressful experience wild primates are subjected to by field researchers (Fedigan, 2010). Although capture is necessary to collar study subjects, obtain morphometric data, or draw blood, the risks of darting wild, arboreal primates are substantial. These risks range from transient elevation of white blood cell counts, to overheating, drug overdose, allergic reaction, pregnancy loss, and fatal injury during a fall (Lambeth et al., 2006; Cunningham et al., 2015). While appropriate veterinary care can mitigate some of the risks of anesthesia to study subjects, seeking alternatives to capture and chemical immobilization are animal welfare imperatives (Glander, 2013; Lane and McDonald, 2010; Osofsky and Hirsch, 2000).

Minimally invasive samples, such as feces or hair, have been used in primate genetics research for nearly three decades (Phillip et al., 1991). Collecting fecal samples poses minimal risk to primate study subjects, and feces can be collected without habituation (Bradley et al., 2001; McGrew et al., 2004). Obtaining DNA from feces is also advantageous to researchers because these materials are more readily available than invasive samples like blood. However, DNA from feces is often very fragmented and upwards of 99% of the DNA can be from gut flora or contaminants (Perry et al., 2010; Perry, 2014; Snyder-Mackler et al., 2016). Thus, studies relying on fecal samples continue to face intrinsic technical challenges, while those using invasive samples have embraced genomics over the last decade (Ryder, 2005; Ekblom and Galindo, 2010; Carroll et al., 2018).

Recent progress has been made moving minimally invasive samples into the genomic age (Perry, 2014; Carroll et al., 2018). Massively parallel sequencers read small sections (~200 bp) of DNA, making the fragmentation inherent in fecal DNA (fDNA) less of an issue than with longer Sanger sequencing reads (Perry, 2014; Perry et al., 2010). Several successful attempts have been made to address the contamination problem by enriching fDNA for target species DNA. Two approaches have obtained target species genomic data from primate feces so far (Perry et al., 2010; Snyder-Mackler et al., 2016; Chiou and Bergey, 2018). The first, DNA bait capture, targets the species of interest's DNA without binding to microbial DNA by using use RNA-baits derived from a reference genome and a microarray (Perry et al., 2010) or from a high-quality sample from the species of interest (Snyder-Mackler et al., 2016). The second, called FecalSeq, enriches for CpG-methylated DNA, which is common in vertebrate genomes and uncommon in bacterial ones (Chiou and Bergey, 2018). This paper will focus on FecalSeq, because it enables enrichment of fecal samples without an accompanying high-quality genome or DNA sample, making it apt for non-model, difficult to sample primates.

After successful enrichment, Chiou and Bergey (2018) used RADseq to prepare samples for sequencing. RADseq sequences areas of the genome near specific restriction enzyme sites throughout the genome (Andrews et al., 2016). While a random sampling of neutral and adaptive alleles from across the entire genome is suitable for many studies (Davey and Blaxter, 2010), RADseq may not be suitable for researchers interested in comprehensive genome-wide scans or local adaptation. Adaptations not covered by the random RAD loci cannot be included; and when signal is detected, it can be difficult to tell if the RAD locus itself is under selection or if it is in linkage disequilibrium (Lowry et al., 2016). Another reduced representation approach, RNAseq, provides results from the exome, but cannot provide information about regulatory regions or provide non-coding areas for comparison (Wang et al., 2009).

Unfortunately, some of the most interesting questions regarding local adaptation and speciation are best answered with a more laborious whole genome approach (Andrews et al., 2016; Lowry et al., 2016). Relatively recent adaptation to local conditions may occur at only a few key loci, which might be missed with a reduced representation approach (Savolainen et al., 2013). Local adaptation along strong environmental gradients often ends in speciation through diversifying selection. When one end of the environmental gradient, or ecotone, selects for traits that would be maladaptive at the other end, these selective pressures can cause speciation with gene flow (Coyne and Orr, 2004; Smith et al., 2011). Neutral gene flow continuing for some time after loci under selection become geographically localized. This tapering of gene flow between speciating populations is an identifying characteristic of ecological speciation relative to allopatric speciation, where all gene flow stops simultaneously (Pinho and Hey, 2010). Reduced representation genomic approaches that exclude neutrally evolving genes, such as RNAseq, cannot discriminate between ecological and allopatric speciation. Thus, whole genomic data are best suited for documenting local adaptation and the process of ecological speciation.

Here I present the case of two species of lemur, the blue-eyed black lemur (*Eulemur flavifrons*) and the black lemur (*E. macaco*). These sister taxa diverged 160 Kya, during the second most recent Pleistocene ice age (Meyer et al., 2015). A date in the Pleistocene supports a hypothesis of speciation in refugia (Markolf and Kappeler, 2013). However, this is not the only plausible hypothesis for how *E. flavifrons* and *E. macaco* diverged.

Despite their narrow geographic ranges, the two species occupy a complex landscape of barriers and selective pressures. Humid, evergreen rainforest in the east rapidly transitions into sub-humid forest and ultimately dry deciduous forest in the west (Schwitzer et al., 2006). Rivers may act as dispersal barriers, and two pockets of forest, or ‘refugia,’ were isolated during glaciation events in the Pleistocene (Vences et al., 2009). The ecological gradient and physical barriers represent an opportunity to understand the relative importance of physical barriers and gradient-driven diversifying selection in primate speciation.

This gradient occurs in the Sahamalaza region of northwestern Madagascar, and it is the only place where the Critically Endangered blue-eyed black lemur occurs. Tree heights can top 30 meters (Schwitzer et al., 2006). Both species are highly arboreal, making the risk of darting-related injury or fatality very high (Birkinshaw, 1999; Volampeno et al., 2011; Cunningham et al., 2015). Further, the terrain in Manongarivo, where the black lemur occurs, makes swift human movement difficult to impossible (*pers. obs.*), making the risk of not catching a darted animal high even outside of Sahamalaza.

Unsurprisingly, genetic studies of these species in the wild are limited. In 1998, Rabarivola et al. examined restriction fragment length polymorphisms to assess the diversity of three populations of black lemur: two insular (Nosy Be and Nosy Komba) and one from the nearby coast (Ambato Peninsula). They found that the population on the larger island, Nosy Be, had the greatest genetic diversity of the three. In 2000, using random amplified polymorphic DNA, Fausser et al. found similar levels of genetic diversity in blue-eyed black lemurs on the Sahamalaza Peninsula as in other lemur species. Finally, an unpublished study of intermediate morphology lemurs at Kapany that assigned them to *E. macaco* relied solely on the mitochondrial D-loop (C. Schwitzer., pers. comm.).

Data on these lemurs derived from captive individuals are more plentiful. Meyer et al. (2015) generated a *de novo* genome assembly for *E. flavifrons* and high coverage genomes for four individuals from each of *E. flavifrons* and *E. macaco*. They identified MITF, a melanocyte development gene, as the gene responsible for *E. flavifrons*’s blue eyes (Meyer et al., 2015).

Whole genome data from wild populations could answer questions about how these species diverged, whether they are locally adapted to the Sahamalaza ecotone, and whether any gene flow is ongoing. In this preliminary study, I demonstrate that whole genomes can be generated from fecal samples collected in the wild.

Methods

From June to Sept 2015, collaborators and I collected 202 fecal samples at 14 sites throughout the ranges of both *E. flavifrons* and *E. macaco* (median: 7.5 samples per site, range: 0-71; Table 2.1; Fig. 2.1). The most intensive sampling efforts focused around the Manongarivo and Andranomalaza (also called the Maitsomalaza) Rivers, where the two species meet. After consulting local conservation experts at Missouri Botanical Garden, Madagascar National Parks, and the Association Européenne pour l'Étude et la Conservation des Lémuriens, I chose additional sampling localities to cover as many remaining populations as possible. We sampled lemurs at Kapany, where an unusual color morph unlike either species was reported (Schwitzer et al., 2005), and Beraty and the northeastern slopes of Manongarivo, where putative hybrids were observed in 1988 (Meyers *et al.*, 1989; Rabarivola *et al.*, 1991).

We also visited Ambodivoahangy, where *E. flavifrons* and *E. macaco* are thought to occur in sympatry (Randriatahina and Rabarivola, 2004). Unfortunately, Ambodivoahangy was the only location where samples were not obtained – there was evidence of intense hunting and we observed no lemurs. We did not continue on to Begiripy, another site of possible sympatry, due to these discouraging findings and security issues. I excluded the southernmost extent of *E. flavifrons'*s range and the Sambirano River Valley based on reports of extensive hunting and no recent observations. Thus, my samples represent many of the remaining populations of both species.

We collected samples by locating the lemurs, usually while they were asleep, with the help of a local guide and then waiting for them to wake up and defecate. As soon as this occurred, we located droppings and picked them up with gloved hands. The entire fecal bolus was collected, with ~3 mL of feces placed in each 15 mL falcon tube. These tubes contained 7.5 mL of RNAlater solution and were shaken vigorously until the contents were homogenized. A Garmin ETrex 10 GPS unit was used to determine coordinates, and tubes were labeled with GPS coordinates, date, and species. We added sex and any unusual physical characteristics (e.g. eye color, ruff length) when we could discern which individual was associated with which sample. This was not always possible as multiple animals in the same tree sometimes defecated simultaneously. In the field, samples were stored in individual baggies at ambient temperatures to prevent any cross contamination from accidental leakage. After October 2015, they were stored in a -20^{o}C freezer.

To extract DNA from fecal samples, I adapted a variation of QIAamp®'s stool kit developed for big cat scat written by American Museum of Natural History researchers Simone Loss Chaves, Isabela Dias, Cristina Pomilla. I then did an AMPure bead cleanup before quantifying total DNA amounts with Qubit Fluorometric Quantification and lemur DNA amounts with qPCR.

For qPCR, I designed a set of primers targeted to methylated areas, because the FecalSeq DNA capture protocol enriches for CpG-methylation. I chose areas of the human genome that were methylated across most human tissues using the ENCODE tracks on the UCSC genome browser (Sloan et al., 2015). I BLASTed several of these areas to my target species' genomes and designed primers that would amplify lemur DNA but not human to avoid identifying any samples potentially contaminated by human collectors as having high levels of target DNA. I also checked that the primers would not amplify anything in the *Escherichia coli* genome using the NCBI primer-BLAST tool (Ye et al., 2012). This practice helped avoid mistaking high quantities of bacterial DNA with high levels of lemur DNA.

I kept the amplicon length between 80-150 bp, and kept the primer melting temperature near 60^{o}C (Ye et al., 2012). I also ensured that the primers were associated with a single copy area of the genome so that copy number variation would not be mistaken for variation in the amount of lemur DNA in a sample. The primers I used were: Forward: 5'-TTTCTGCCTCGCGTATCCC-3' and Reverse: 5'-ATCGCCCCTTTGGTTCGC-3'. I ran each sample for only one amplicon and in duplicate to conserve DNA for downstream work. Thus, I did not treat the qPCR results as actual quantitation and instead used them to compare samples to each other and to themselves, before and after enrichment.

Samples that had either a minimum estimate of 200 ng total fDNA from Qubiting or 0.5% ng total lemur DNA according to the qPCR were moved onto FecalSeq enrichment (Chiou and Bergey, 2018). After FecalSeq, samples were requantified with Qubit and qPCR. I expected successfully enriched samples to have lower total DNA amounts and higher proportions of lemur DNA than they did previously. Samples that enriched successfully were prepared for sequencing using a Nextera DNA kit and a protocol developed by Kenneth Chiou and Noah Snyder-Mackler for very small DNA inputs. Fifty-nine samples were sequenced on 4 Illumina MiSeq lanes. These results were mapped to the well-characterized gray mouse lemur genome (*Microcebus murinus*; Larsen et al., 2017). The eight samples with the best mapping and lowest duplication rates were pooled and sequenced on two Illumina HiSeq X lanes (Table 2.2).

Lab work from extraction through FecalSeq DNA capture and post-FecalSeq quality checks was performed at the American Museum of Natural History. Library preparation was performed at the Snyder-Mackler Lab at the University of Washington by Kenneth Chiou. Samples were sequenced at the UW core. The entire step-by-step protocol, from frozen samples to FecalSeq, is available in Supplementary File 2.1. As a preliminary assessment of the utility of these data, single nucleotide polymorphisms (SNPs) were identified using the mpileup command in SAMtools (Li et al., 2009).

Results

Samples had a median of 273 ng DNA each after extraction. I selected 88 samples covering all field sites for FecalSeq enrichment, with a median DNA quantity of 356 ng. After FecalSeq, 59 samples had quantifiable amounts of DNA (median = 0.75 ng). These 59 samples had a median of 6887.5 mapped reads, with median PCR duplication rates of 8.33% and median mapping rates of 55.68%. The eight best samples, their reads, mapping and duplication rates are described in Table 2.2. Filtering SNPs for quality and coverage yielded 41,374 reliable SNPs. Of these, forty-two were high impact mutations, most of which were stop gains.

Discussion

While this preliminary investigation certainly leaves more questions than answers about the black lemurs, it does indicate that whole genomes can be obtained from non-invasively collected samples. As additional samples are sequenced, I look forward to investigating local adaptation, population genetic diversity, and whether introgression between *E. macaco* and *E. flavifrons* has occurred. Even so, this application of DNA capture methods is a first for extant lemurs, for arboreal primates, and for any wild population in Madagascar.

These results demonstrate the feasibility of obtaining genomic data from low-quality fecal samples. Indeed, my samples had lower quantities of fDNA and target DNA at every point in analyses than either of the previous studies that obtained genomic data from wild-collected baboon feces (Snyder-Mackler et al., 2016; Chiou and Bergey, 2018). This difference may have been related to storage conditions or the difference in diet between baboons and black lemurs (Panasci et al., 2011). Nevertheless, I was able to obtain sequence from nearly half the samples I extracted. This success with extremely low quantity and quality DNA samples indicates that other studies using minimally invasive samples need not default to RADseq or RNAseq when a whole genome approach would suit their research questions better.

One benefit of this pipeline is that species-specific reference genomes are not strictly necessary for primates or other organisms with a reasonably close relative that does have its own reference genome. Nor is high-quality, uncontaminated DNA necessary. The qPCR step with species-specific primers and the high-quality DNA dilutions can be omitted, although this is not ideal. One could instead assume that the concentration of target species DNA in a fecal sample is 1% of the total DNA quantity (prior to FecalSeq enrichment) using Qubit values alone (Perry et al., 2010; Snyder-Mackler et al., 2016). This assumption could lead to wasted time and reagents if the samples are highly variable in their target DNA concentrations and is not recommended as a first step. However, this protocol is not a necessary or sensible choice for well-studied organisms for which blood or tissue is readily available.

Despite the promising progress in minimally invasive conservation genomics in the last few years, darting and collecting blood is still cheaper, quicker, and more likely to yield results than fecal samples (Perry et al., 2010; Snyder-Mackler et al., 2016; Chiou and Bergey, 2018). Even considering the expense of veterinary care during darting, those costs are currently outweighed by the risk of low target DNA in feces, the extensive processing needed prior to library prep, and the much greater sequencing depth required to approximate uncontaminated samples even after DNA capture. However, when we look at only the prices of lanes on a sequencer, we fail to account for the risks to arboreal primates that invasive sample collection poses (Cunningham et al., 2015; Glander, 2013; Lane and McDonald, 2010; Osofsky and Hirsch, 2000). The possibility of obtaining whole genomes from feces should inspire other researchers who are interested in moving from genetics to genomics without sacrificing their study subjects' welfare.

Table 2.1. Fecal samples collected for this study. Field site numbers correspond to those in Figure 2.1.

Field Site Name		Field Site Code	Species	Number of individuals sampled
1.	Ambodimanga	AB	***E. flavifrons***	7
2.	Mahevatanana	MA	***E. flavifrons***	3
3.	Sahamalaza	SA	***E. flavifrons***	71
4.	Ambodivohangy			0
5.	Andranomatavy	AM	*E. macaco*	10
6.	Antafiabe	AN	*E. macaco*	8
7.	Beraty	BE	*E. macaco*	6
8.	Bongomaravavy	BG	*E. macaco*	5
9.	Galoko	GA	*E. macaco*	7
10.	Kapany	KP	*E. macaco*	45
11.	Kolobinono	KL	*E. macaco*	1
12.	Nosy Be	NB	*E. macaco*	11
13.	Nosy Komba	NK	*E. macaco*	18
14.	Manongarivo	MG	*E. macaco*	10
TOTAL				**202**

Table 2.2. Mapping and duplication rates for whole lemur genome sequencing from fecal-derived DNA.

Sample ID	Paired reads	Total reads	Mapped reads	Mapping rate (%)	Duplication rate (%)	Coverage (X)
BT02	96936964	193873928	115581007	59.44	39.63	3.488813
BE05	116337188	232674376	166074413	70.28	72.92	2.248648
KP02	73238135	146476270	126538543	83.99	84.01	1.011676
AM04	98951152	197902304	118657782	59.74	60.65	2.334592
BE03	116651555	233303110	169454385	71.79	72.23	2.352874
BE04	92426124	184852248	106402476	56.75	58.16	2.22594
NB11	125732457	251464914	167381150	66.51	70.38	2.478915
MA06	80589878	161179756	98900679	61.2	63.82	1.789113
MEDIAN				**63.86**	**67.10**	**2.29162**

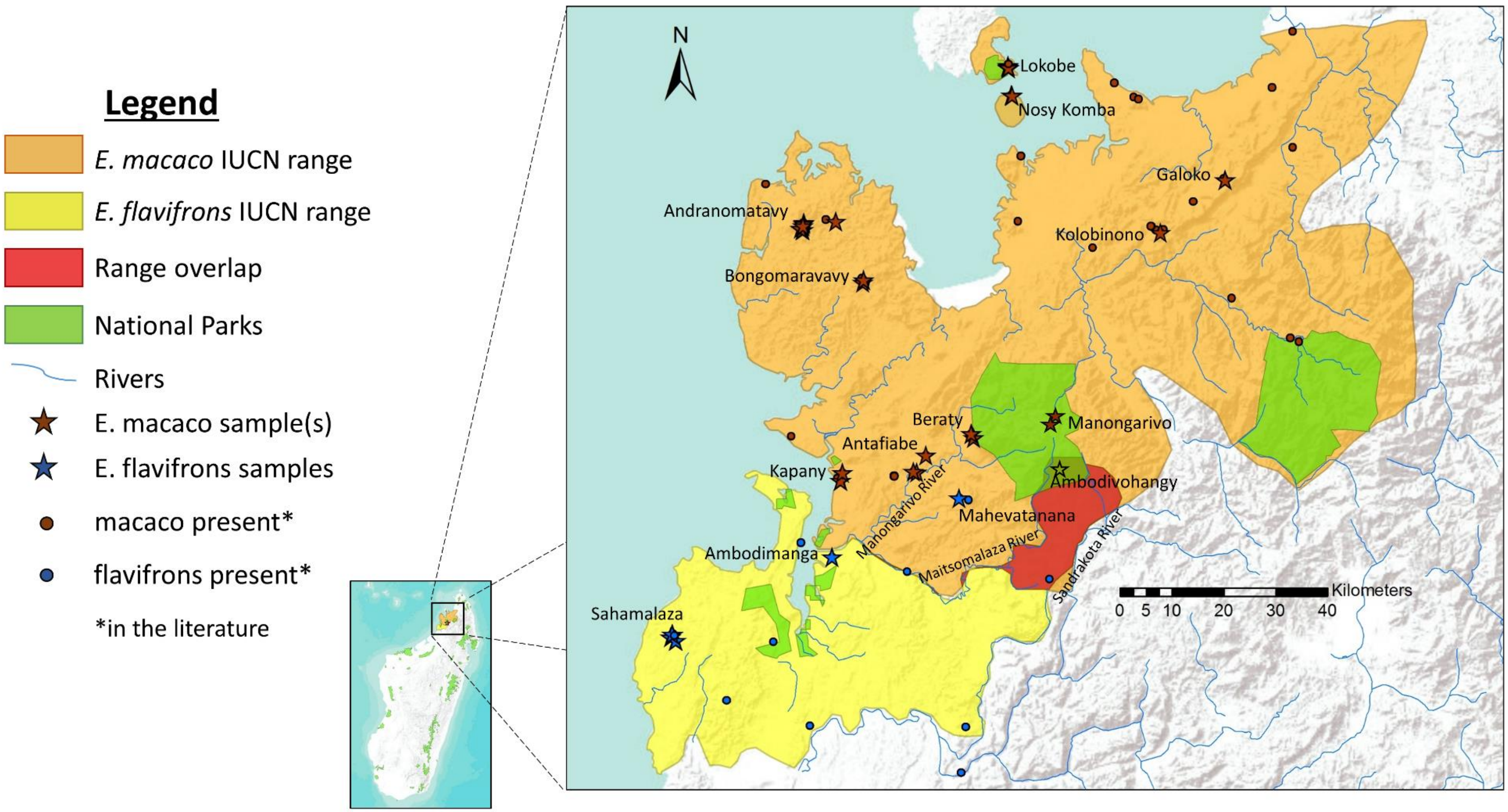


Figure 2.1. Map of sampling localities for the blue-eyed black lemur (*Eulemur flavifrons*) and the black lemur's (*E. macaco*) throughout their ranges in northwestern Madagascar.

Chapter 3: Modeling arboreal species' distributions in degraded forests: lessons from the lemurs of Madagascar

Abstract

Identifying where threatened species occur is a basic necessity of conservation biology. While many tools like ecological niche modeling can help accomplish that goal, there are fewer methods for integrating our knowledge of species' fundamental biotic needs and their sensitivity to habitat disturbance. In this study, I determine how forest loss and degradation limit the habitat available to Madagascar's lemurs, the world's most threatened mammals. Using niche modeling, a high-quality forest cover map, and an extensive collection of curated occurrence records, I demonstrate that lemurs lost 29% of their habitat to forest loss and degradation from 1973 to 2014. Over half of species were most negatively affected by proximity to the forest edge, with mean forest patch size mattering less than edge effects. While Madagascar's protected area system covers most lemur species, nearly half of extant lemur habitat is unprotected. Two species, Jolly's mouse lemur (*Microcebus jollyae*) and Grewcock's sportive lemur (*Lepilemur grewcockorum*), do not occur in any protected area. Further, *M. jollyae* lost 95% of its habitat to forest loss and fragmentation in a 31-year period, indicating urgent need for conservation measures. I suggest reforestation efforts as an intervention for these species, and highlight NGO- and community-managed protected areas, including Beanka, Tsimembo Forest, Ranobe PK 32, and Amoron'i Onilahy, as highly suitable areas for lemurs that should be targeted for additional funding and support.

Keywords

Forest fragmentation, forest cover, anthropogenic disturbance, lemurs, Madagascar, ecological niche modeling

Introduction

Lemurs are the most endangered group of mammals, with 94% of species facing extinction (Schwitzer et al., 2014). These arboreal primates are threatened by forest loss and fragmentation, but lemurs vary in their sensitivity to habitat degradation (Irwin et al., 2010; Arroyo-Rodríguez et al., 2013). Studies of lemur responses to forest degradation frequently compare conspecifics in nearby forest parcels of different quality or size (Ganzhorn, 1995; Herrera et al., 2011; Merenlender et al., 1998; Sawyer et al., 2017; Steffens and Lehman, 2018). These case studies provide crucial information on lemur presence, density, health, and/or behavioral changes across habitat types, but these comparisons can be quite difficult. Study sites differ in their size, levels of degradation, time since the last logging event, levels of ongoing disturbance, and even in their definitions of degradation (Arroyo-Rodríguez et al., 2013; Irwin, 2016). Thus, it can be quite difficult to make inferences about whether unstudied forests are suitably intact for lemurs, even before different species' ecological needs are considered.

One trend supported by evidence from multiple forest types is that frugivorous species struggle more than folivores in degraded habitat. Frugivores need larger home ranges and bigger trees to meet their nutritional requirements than folivores do (Lehman, 2007; Irwin et al., 2010). True lemurs with fruit-heavy diets, including *Eulemur collaris, E. flavifrons*, and *E. rufus* had lower densities near the edges of the forests in which they were studied (Lehman, 2007; Schwitzer et al., 2007; Donati et al., 2011).

Some folivorous species even increase their population density as regrowth commences after disturbance (Irwin, 2008). Several species that can subsist on leaves, including *Propithecus diadema, Hapalemur griseus*, and *Avahi laniger*, had the same or greater densities near the edge or in fragments (Lehman et al. 2006c; Irwin, 2008). *Microcebus rufus*, an insectivore, also increased in density in a degraded habitat, perhaps because recent tree clearing increased its food supply (Lehman et al., 2006a, 2006c; Herrera et al., 2011). These changes may not be sustainable, because living near the forest edge means living closer to humans. Hunting is more common further away from the forest core (Irwin et al., 2010; Schwitzer et al., 2011). Some lemurs are also more likely to have parasites in degraded forest, smaller fragments, and nearer the forest edge (Wright et al., 2009; Ragazzo et al., 2018; Raharivololona and Ganzhorn, 2009). Long-term, lemurs in forest fragments and nearer the forest edge have lower genetic diversity than conspecifics in core primary forest (Craul et al., 2009; Radespiel et al., 2018).

However, one cannot assume that species with similar traits, even closely related ones, will respond to habitat degradation in the same ways. The densities of *Avahi peyrierasi*, *Hapalemur aureus*, and *Prolemur simus*, all folivores, decreased in disturbed forest, while frugivores, *Eulemur rubriventer and E. fulvus* were unaffected (Herrera et al., 2011). In an unusual study that directly compared sympatric congeners in the same forest, *Microcebus murinus* and *M. ravelobensis* had almost completely different responses to the forest edge. At Ankarafantsika National Park, Burke and Lehman (2014) captured 82% of *M. murinus* in the interior and 72% of *M. ravelobensis* at the forest edge. Thus, we cannot assume that unstudied species will exhibit responses to habitat degradation similar to those of their close relatives for which data are available.

Identifying not just forested, but high-quality, intact habitat at a regional scale requires a supplemental approach to fragmentation case studies. Ecological niche models can be used to map where suitable areas occur throughout a landscape by comparing georeferenced occurrence records with environmental variables like precipitation and temperature (Kremen et al., 2008; Mulligan, 2010; Brown and Yoder, 2015). However, these models typically include only climate as a predictor because of the difficulty in including anthropogenic habitat loss and degradation (Fahrig, 2003; Irwin et al., 2010; Arroyo-Rodríguez et al., 2013). Climate-based models can make use of species occurrence records from the past several decades. Indeed, many papers include records from museum collections because recent climate data still provide relevant information about specimens' environments (Hijmans et al., 2005; Wilmé et al., 2006; Aschroft et al., 2011). Human-driven forest change, by contrast, has proceeded rapidly in biodiversity hotspots and requires study at a much finer temporal scale than the 30-year window used to define climate (Brooks et al., 2002).

From 1953 to 2014, Madagascar lost 44% of its forest cover with losses accelerating from 2005 onward. The remaining forest is increasingly subject to human disruption – in 1973 a quarter of all forest was within 100 meters of the forest edge. Today, 49% of forest is within 100 meters of the edge (Vieilledent et al., 2019). Accounting for this rate of landscape change within the traditional niche modeling context presents some undesirable options: one could violate model assumptions by including forest predictor variables from a time frame associated with only some of the species records (Warren and Seifert, 2011; Merow et al., 2013). Alternatively, one could discard years' worth of occurrence data to accommodate the narrow time window associated with a forest cover variable.

In this chapter, I demonstrate a post hoc method for limiting climate-based niche models to intact forest. I develop species-specific thresholds for habitat degradation using presence data from 60 years of literature on Madagascar's lemurs and remotely sensed forest cover. By using the same approach for over one hundred species, I identify which species have been the most affected by both forest loss and degradation. I also use these forest-aware niche models to identify species that have no protected habitat and to highlight areas that are suitable to many species of lemurs outside of Madagascar's protected area system.

Methods

I obtained GPS occurrence records for all extant species of lemur identified in peer-reviewed literature from three types of sources (Supplementary Table 3.1). The first were points I collected in the field and points courtesy of other researchers. Second were online databases including the Global Biodiversity Information Facility (GBIF), Mammal Networked Information System (MaNIS), VertNet, and Reseau de la Biodiversité de Madagascar (ReBioMa). Third, I recorded localities from 277 published sources.

All articles published in Lemur News, Madagascar Conservation and Development, and Primate Conservation were visually scanned for GPS coordinates. I also conducted several Google Scholar searches using each of the following search terms combined with "lemur": "GPS," "occurrence," "coordinates," and "range" (e.g. "lemur" AND "GPS"). I visually scanned articles for coordinates from these results and recorded the points. Points were traced to their original publication whenever possible, and the year of first publication was recorded. These papers, the databases, and field efforts yielded 26,197 records (Supplementary Table 3.1).

All points were plotted and evaluated, and suspect data (*e.g.* undated localities for species that have been split taxonomically) were removed from the dataset, leaving 17,180 reliable and unique locality records (Supplementary Table 3.2). These points were then thinned to reduce the effects of spatial autocorrelation and sampling bias (Kramer-Schadt et al., 2013; Boria et al., 2014). To balance the need to correct for bias and the desire to include micro-endemic taxa in this study, points were thinned to no closer than 5 km for species with IUCN polygons ≥5,000 km^2, no closer than 2.5 km for species with IUCN polygons between 5,000 km^2 and 2,500 km^2, and no closer than 1 km for species with polygons <2,500 km^2 and for species not assessed by IUCN yet, similar to Brown and Yoder (2015). Only species that had at least 5 occurrences after thinning were analyzed (Pearson et al., 2007). 104 species of lemur met this minimum.

Climate variables related to temperature, precipitation, and solar radiation, and soil variables are detailed in Table 3.1. They represent a 30-year window of very recent climate, centered on 1985, and were downloaded from the WorldClim, CliMond, SoilGrids, and WorldGrids databases (Hijmans et al., 2005; Kriticos et al., 2014). Variables included in analyses had Pearson's r correlation coefficients < |0.85| (Merow et al., 2013).

I constructed niche models in Maxent 3.4.1 (Phillips et al., 2017). Model parameters were selected for each species using ENMeval 0.2.2 (Muscarella et al., 2014). Because many species had a small sample size after thinning, I chose from models with a ΔAIC ≤ 2, a method that improves models built with few occurrence records (Galante et al., 2014). Of those, I selected the parameters that had the mean tenth percentile omission rate closest to 0.1 to limit over- and under-fitting, and then the highest mean AUC (Warren and Seifert, 2011; Muscarella et al., 2014). Model parameters and quality metrics are available in Supplementary Table 3.3.

A maximum of ten thousand pseudo-absence points, no more than one per cell, was drawn from a species-specific buffer of 50 km, drawn around all occurrence records for each species (Phillips et al., 2009). Models were then projected from this background to all of Madagascar. I assigned locations with climate outside the range of the training background a suitability score of zero (Elith et al., 2011). I used a 10% training threshold, which is the suitability score associated with the 10th percentile presence record, to turn models into a binary prediction of 1: suitable climate, or 0: unsuitable.

Forest quality metrics were calculated from forest layers at six time points generated by Vieilledent et al. (2018). These layers were resampled to 1 km^2 resolution. Forest cover at this resolution was highly correlated across all six time points, and no interval had a Pearson's r correlation coefficient < |0.85| (Table 3.2).

Four metrics of habitat integrity, selected for their relevance to lemurs and interpretability, were used in this study. Mean Patch Area was included because patch size influences lemur presence, density, and behavior (Irwin, 2007, 2016; Schüßler, 2018). Edge Density and minimum Distance to Edge were chosen because of lemurs' varied responses to forest edge (Donati et al., 2011; Lehman et al., 2006a, 2006b, 2006c; Lehman, 2007; Schwitzer et al., 2007; Herrera et al., 2011). Finally, Mean Perimeter-Area Ratio was chosen to represent possible interactions between perimeters/edges and patch size.

These metrics are defined in Fragstats software and were calculated in the R package SDMTools (McGarigal et al., 2002; VanDerWal et al., 2012). Edges were defined as where forest and non-forest matrix meet, and patch as forest identified in the Vieilledent et al. (2018) layers. For each occurrence record, habitat integrity values were extracted from the forest layers closest in time to its publication year (Table 3.2). Only points with known publication or collection dates were included in these analyses, excluding 154 records. For each species, the most disturbed value at an occurrence record was determined for each metric and assumed to represent the most habitat degradation that species can tolerate. This assumed that lemurs would be more visible to researchers in disturbed habitat and that occurrence records would therefore include lemurs in the most degraded habitat they are able to occupy.

These species-specific minimum habitat integrity values were used to identify forest ≥ that minimum value for the most recent year available, 2014, and for the earliest year in the Vieilledent et al. (2018) dataset covered by the "current" climate data, 1973. Suitable forest was defined as including only pixels for which the minimum value was met for all four habitat integrity metrics. I chose this conservative consensus approach because I did not want to count cells as false positives if a species is highly susceptible to the effects of only one metric – for example, a species that is edge tolerant but requires a large patch size might have an overestimated suitable area if MPA is excluded. Further, due to spatial autocorrelation, low-quality forest pixels are likely to be disturbed across multiple metrics.

Suitable forest was identified for 103 species – *Hapalemur alaotrensis* was excluded because they are arguably marsh-dwellers (Guillera-Arroita et al., 2010), and the Lac Alaotra marshes are not included in the forest cover dataset. These fragmentation tolerance maps and the most recent forest cover map (Vieilledent et al., 2018) were used to limit individual species' climate-based niche models to only pixels that were climatically suitable, forested, and sufficiently intact. Areas of suitable habitat were calculated from the niche model alone, from the model limited to only forested land, and from the model limited to sufficiently intact forest using the habitat integrity metrics. These distinctions allowed me to calculate the percentage of habitat lost specifically to habitat degradation, in addition to outright forest loss, for each of the 103 species.

Protected area (PA) data were downloaded from ReBioMa and sorted into two categories: managed by Madagascar National Parks (e.g. special reserves, strict nature reserves) or less protected PAs including community-managed areas and those managed and funded by NGOs under the auspices of the Ministry of Environment, Ecology and Forests (Virah-Sawmy et al., 2014; Gardner et al., 2018). These data were used to identify areas suitable to high levels of lemur diversity not currently under protection. Pixels were identified as being "highly suitable" if they were in the 90th percentile or above for number of species that could occupy the area. This threshold was calculated separately for each ecoregion since different types of forest support different numbers of species (Muldoon and Goodman, 2010).

Results

In 1973, Madagascar had 286,966 km^2 of forested land that was suitable for at least one lemur species based on their climatic niches (Fig. 3.1). Of this area, 92% was sufficiently intact to support at least one species of lemur. By 2014, 258,687 km^2 of climatically suitable areas were forested, representing a 10% decline due to forest loss in 31 years (Figs. 3.1, 3.2). Only 203,940 km^2 of this forested area were sufficiently intact to support any lemurs, rendering 21% of the forested area unsuitable. From 1973 to 2014, Lemuroidea lost 29% of their habitat to forest, loss, fragmentation, and degradation (Figs. 3.1, 3.2).

Individual lemur species' responses to forest conversion varied greatly. From 1973 to 2014, each species lost an average of 51% of its otherwise suitable habitat to forest loss and degradation. These reductions ranged from 10% (*Propithecus candidus* and *Lepilemur petteri*) to more than 90% (*Lepilemur ahmansonorum*, 91%; *Microcebus jollyae*, 95%; and the Manombo mouse lemur, *Microcebus* sp. nova 1, 96%). Individual species' habitat areas and losses appear in Table 3.3. The habitat available to three example species based on climate only, forest cover, and fragmentation tolerance are shown in Fig. 3.3.

For over half of species (56 out of 103), Distance to Edge caused the greatest amount of habitat loss (Table 3.3). On average, a too-close forest edge reduced suitable areas by 26% when considered alone. Mean Perimeter-Area Ratio, Edge Density, and Mean Patch Area each eliminated on average 17%, 14%, and 12% of forested habitat, respectively.

Nearly all lemur species had some suitable habitat in a PA, with the exceptions of *Microcebus jollyae* and *Lepilemur grewcockorum* (Table 3.3). On average, 48% of lemurs' remaining habitat occurred in some kind of PA. When only areas managed by Madagascar National Parks (MNP) were considered, this number fell to 18%. In total, 15,675 km^2 of lemur habitat was protected by MNP, and an additional 35,417 km^2 have a lower level of protection. Habitat protected by MNP was suitable to more species of lemur (11.8 on average) than that in other types of PAs (10.8 on average) and unprotected habitat (5.6 on average). However, not all areas that can support high lemur biodiversity are protected. Figs. 3.4, 3.5, and 3.6 illustrate areas throughout Madagascar that are highly suitable to lemurs but are currently unprotected or have lower levels of protection.

Discussion

Lemurs lost over a quarter of their remaining habitat to forest loss and degradation in a 31-year period. Forest degradation alone resulted in lemurs losing 21% of their habitat from 1973 to 2014 (Fig. 3.2). Degradation more than doubled habitat reduction due to forest loss (10%), which is often recognized as the greatest threat to primates (Fig. 3.2; Fahrig, 2003; Irwin et al., 2010; Arroyo-Rodríguez et al., 2013; Estrada et al., 2017). With forest being lost at a rate of 1.9%/year, all of Madagascar's tropical forest could disappear in the next fifty years

(Vieilledent et al., 2019). If habitat degradation continues to outpace forest loss, lemurs could disappear even sooner.

Lemurs are particularly sensitive to the forest edge (Table 3.3). When considered on its own, being too close to the forest edge rendered a quarter of forested areas unsuitable. Distance to edge was the single biggest driver of habitat lost to forest degradation of the metrics considered for over half of species. These results are a little surprising, because several studies have demonstrated that folivores are not negatively affected by the forest edge or that they even prefer it (Lehman, 2007; Irwin, 2008; Irwin et al., 2010). Food availability for certain dietary guilds, including folivores and insectivores, increases where recent forest disturbance has occurred (Lehman et al., 2006a, 2006c; Herrera et al., 2011). Thus, one might have expected forest size to matter more, since its effects seem more uniformly negative. However, mean patch area rendered less forest unsuitable than any of the edge-related metrics (Irwin et al., 2010; Irwin 2016).

The fact that proximity to the nearest forest edge is such a strong indicator of lemur absence probably represents the negative influence of human contact. Threats like hunting, artisanal mining, logging, and livestock incursions are more present near the forest edge (Irwin et al., 2010; Schwitzer et al., 2014). Temporarily increased food supply may not be enough to offset these factors for lemurs.

These habitat losses due to edge proximity underscore the need for protecting Madagascar's remaining intact forests. In 2014, the most recent year for which data were available, the mean distance to edge in Malagasy forests was 300 m and falling (Vieilledent et al., 2018). Promoting forest integrity by reducing deforestation rates is therefore critical to lemur survival. Madagascar's network of protected areas (PAs) counts habitat protection amongst its goals, and this study shows that half of lemurs' remaining habitat has some level of official protection.

Most of this protected lemur habitat is in PAs created after 2003, when the Malagasy government oversaw a quadrupling of protected lands in Madagascar. While PAs created prior to 2003 had biological research and conservation as their primary focus, the hurried timeline for PAs created after 2003 and understanding of the rights of rural people made Strict Nature Reserves, Madagascar's highest level of protection for biodiversity, infeasible. Thus, these new-style PAs are often multi-use areas, where an international NGO and the local community share management of the site. These areas permit resource extraction and provide less protection to lemur habitat on paper and in practice than PAs managed by MNP (Virah-Sawmy et al., 2014; Gardner et al., 2018).

Several of these modern PAs are suitable to high levels of lemur biodiversity. In the western dry forest ecoregion, the Mahavavy Kinkony Complex, Beanka PA, Tsimembo Forest, and Menabe Antimena Forest are all species-rich and have sufficiently intact forest for lemurs (Fig. 3.4). In the southwestern spiny forest, highly suitable habitat is found in the western part of Ranobe PK 32, the Amoron'i Onilahy PA along the Onilahy River, and the PAs adjoining Andohahela National Park (Fig. 3.5). In the eastern humid forests, the Corridor Forestier Ankeniheny Zahamena (CAZ), Marolambo, and the northeastern arm of Makira Natural Park are similarly diverse and intact (Fig. 3.6). Most of these sites were included in the Lemur Action Plan, an emergency site-based conservation and fundraising effort for lemurs started in 2013 (Schwitzer et al., 2013). Should a revised plan be issued, Beanka, Tsimembo Forest, Ranobe PK 32, and Amoron'i Onilahy merit consideration for inclusion and for increased support of the community-led efforts at these sites.

Similarly, the completely unprotected areas at Anosibe An'Ala in the east, between the Mamambolo and Tsiribihina Rivers in the west, and south of the Onilahy River/east of Beza Mahafaly in the south are suitable to many different lemur species. If the Malagasy government continues its PA expansion, these relatively unfragmented areas might pose the least disruption to local livelihoods and the best hope for lemurs.

Work outside of PAs is needed, if the goal is to prevent any species of lemur from going extinct. Two species are missing from Madagascar's protected area system: Grewcock's sportive lemur (*Lepilemur grewcockorum*) and Jolly's mouse lemur (*Microcebus jollyae*). *L. grewcockorum* lost 75% of its habitat to forest degradation in three decades, and *M. jollyae* lost a shocking 95% (Fig. 3.3). Similarly, the Manombo mouse lemur (*M.* sp.nova 1), which occurs in the Alan'Agnalazaha PA, lost 97% of its habitat and may go extinct before it is even named. Urgent action, such as targeted reforestation efforts aimed at putting more distance between these lemurs and the forest edge, are necessary to ensure these species' survival. The successful community-based reforestation and agroforestry project at Kianjavato, an unprotected site funded by conservation NGO the Madagascar Biodiversity Project, could serve as an example (Manjaribe et al., 2013).

This study highlights the need to consider not just forest cover, but also forest quality, in arboreal species conservation (Irwin, 2016; Schwitzer et al., 2011). The methods outlined here are applicable to other systems where occurrence records and satellite maps of forest cover are available. Regional scale models that omit forest quality data may overestimate the amount of habitat available to species of interest when scaled up, which could hamper conservation planning (Arroyo-Rodríguez et al., 2013; Irwin, 2016).

Certainly, as far as lemurs are concerned, not all forest is equal. The increasingly fragmented nature of Madagascar's forests has drastically reduced the habitat available to these threatened animals and brought lemurs into closer contact with humans than ever before. Much of lemur's remaining habitat is in PAs jointly-managed by locals and international conservation organizations. Maintaining our commitments to those communities is crucial as Madagascar as it works to make its PAs more effective and ensure a future for lemurs and people alike.

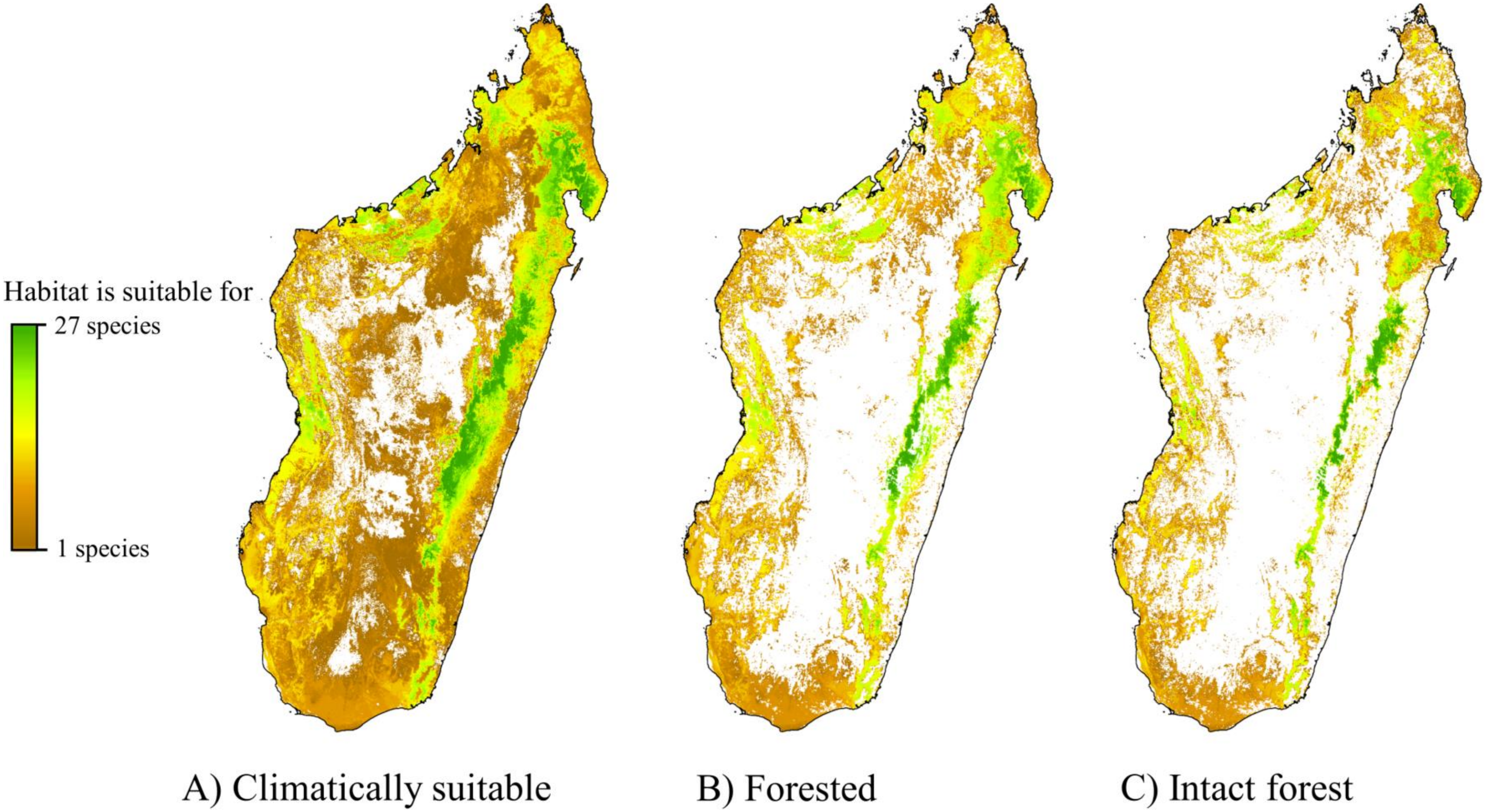
Table 3.1. Environmental variables included in niche model construction.

Climate Variable	**Source**
BIO2: Mean diurnal temperature range	WorldClim v1.4; (Hijmans et al., 2005)
BIO3: Isothermality	WorldClim v1.4; (Hijmans et al., 2005)
BIO4: Temperature seasonality	WorldClim v1.4; (Hijmans et al., 2005)
BIO5: Max temperature of warmest month	WorldClim v1.4; (Hijmans et al., 2005)
BIO6: Min temperature of coldest month	WorldClim v1.4; (Hijmans et al., 2005)
BIO12: Annual Precipitation	WorldClim v1.4; (Hijmans et al., 2005)
BIO13: Precipitation of Wettest Month	WorldClim v1.4; (Hijmans et al., 2005)
BIO16 Precipitation of wettest quarter	WorldClim v1.4; (Hijmans et al., 2005)
BIO17: Precipitation of driest quarter	WorldClim v1.4; (Hijmans et al., 2005)
BIO18: Precipitation of warmest quarter	WorldClim v1.4; (Hijmans et al., 2005)
BIO21: Highest weekly solar radiation	CliMond v1.2; (Kriticos et al., 2014)
BIO22: Lowest weekly solar radiation	CliMond v1.2; (Kriticos et al., 2014)
BIO24: Radiation of wettest quarter	CliMond v1.2; (Kriticos et al., 2014)
BIO25: Radiation of driest quarter	CliMond v1.2; (Kriticos et al., 2014)
Slope	WorldGrids; (Becker et al., 2009; Amante and Eakins, 2009)
Topographic wetness	WorldGrids; (Becker et al., 2009; Amante and Eakins, 2009)
Depth to bedrock	SoilGrids v0.5; (Hengl et al., 2017)
Soil acidity (pH in H_2O)	SoilGrids v0.5; (Hengl et al., 2017)
Soil organic carbon content	SoilGrids v0.5; (Hengl et al., 2017)

Table 3.2. Forest cover years from which fragmentation metrics were generated. The second column indicates the Pearson's correlation values for chronologically adjacent forest cover layers. The years for which a fragmentation layer was used to assign values to an occurrence record, and the number of lemur records falling in each interval are also shown.

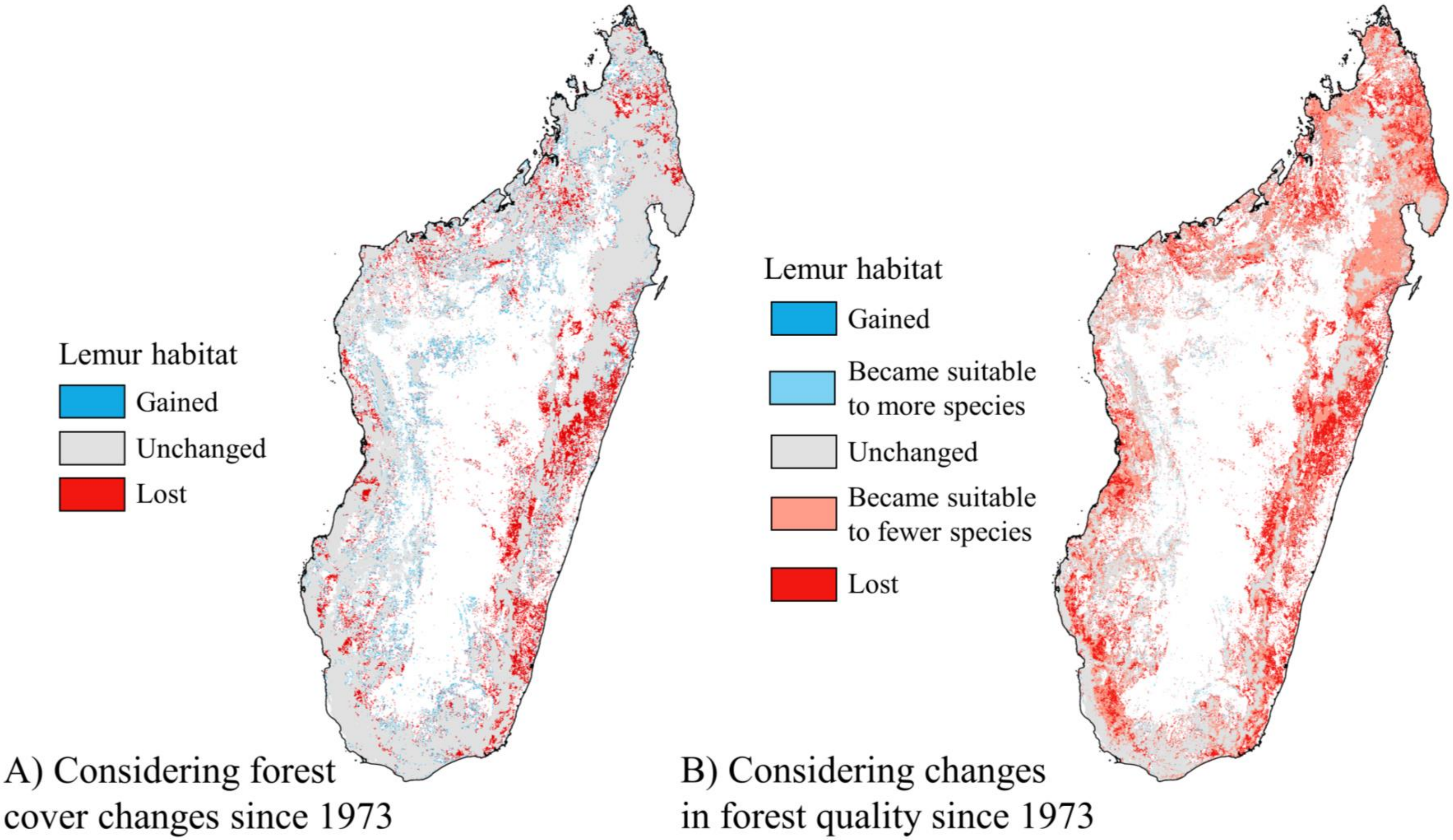
Forest cover layer	Correlation to previous layer (Pearson's r)	Interval covered by each forest cover layer	Lemur occurrence records in the interval
1973	NA	Between 1963 and 1982	126
1990	0.862	Between 1983 and 1995	179
2000	0.995	Between 1996 and 2003	1649
2005	0.996	Between 2004 and 2008	2064
2010	0.994	Between 2009 and 2012	2237
2014	0.993	2013 onwards	2487

Table 3.3 is available in an attached Excel file. Areas in square kilometers of lemur habitat limited to forest cover in 1973 and in 2014. Habitat areas once lemur sensitivity to forest degradation is accounted for are also listed for 1973 and 2014. The percent of habitat rendered unsuitable by forest fragmentation is also listed, as is the broad ecoregion each species was designated to for analysis. The last four columns show how each individual fragmentation metric (edge density, distance to edge, mean patch area, and mean perimeter-area ratio) affected the area of suitable habitat available to each species. These areas represent losses in addition to outright forest loss. Redder cells indicate greater habitat losses for a species caused by their response to that particular degradation metric.



A) Climatically suitable B) Forested C) Intact forest

Figure 3.1. Maps of Madagascar indicating where suitable habitat for at least one species of lemur occurs, considering: A). climatic niches only, B). remaining forest cover, and C). lemurs' tolerance of forest degradation. Greener areas indicate the habitat is suitable to more species. These maps do not account for physical barriers or competition between species.



A) Considering forest cover changes since 1973

B) Considering changes in forest quality since 1973

Figure 3.2. Lemur habitat changes from 1973 to 2014, A). considering only forest cover, and B). considering species-specific requirements for intact forest.

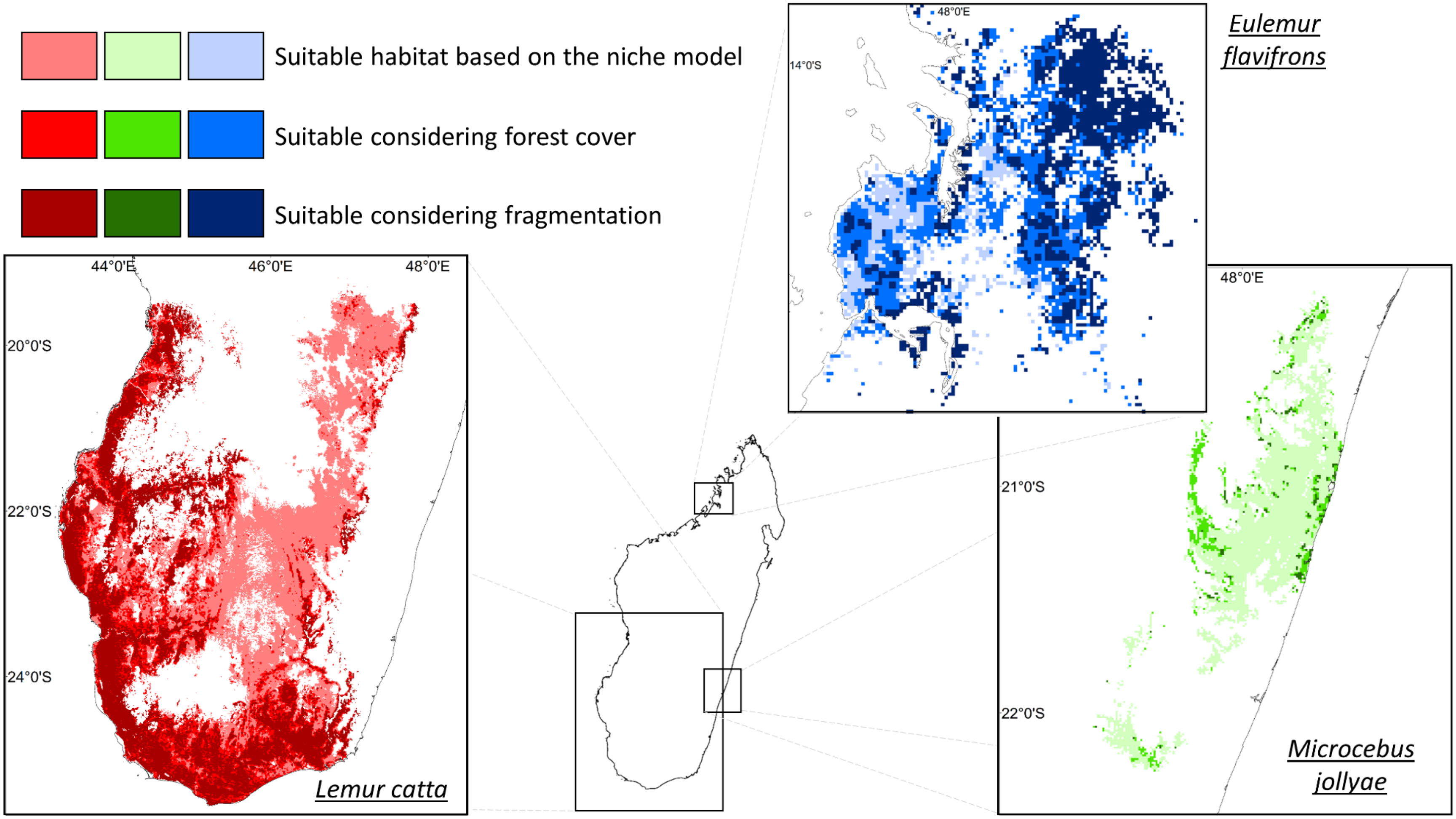


Figure 3.3. Potentially suitable areas for three species (from left to right: *Lemur catta, Eulemur flavifrons,* and *Microcebus jollyae*) when their climactic niche model, forest cover, and their individual sensitivity to forest fragmentation are considered. *M. jollyae* has lost 96% of its available habitat to forest loss and degradation and is one of two lemur species with no habitat under protection.

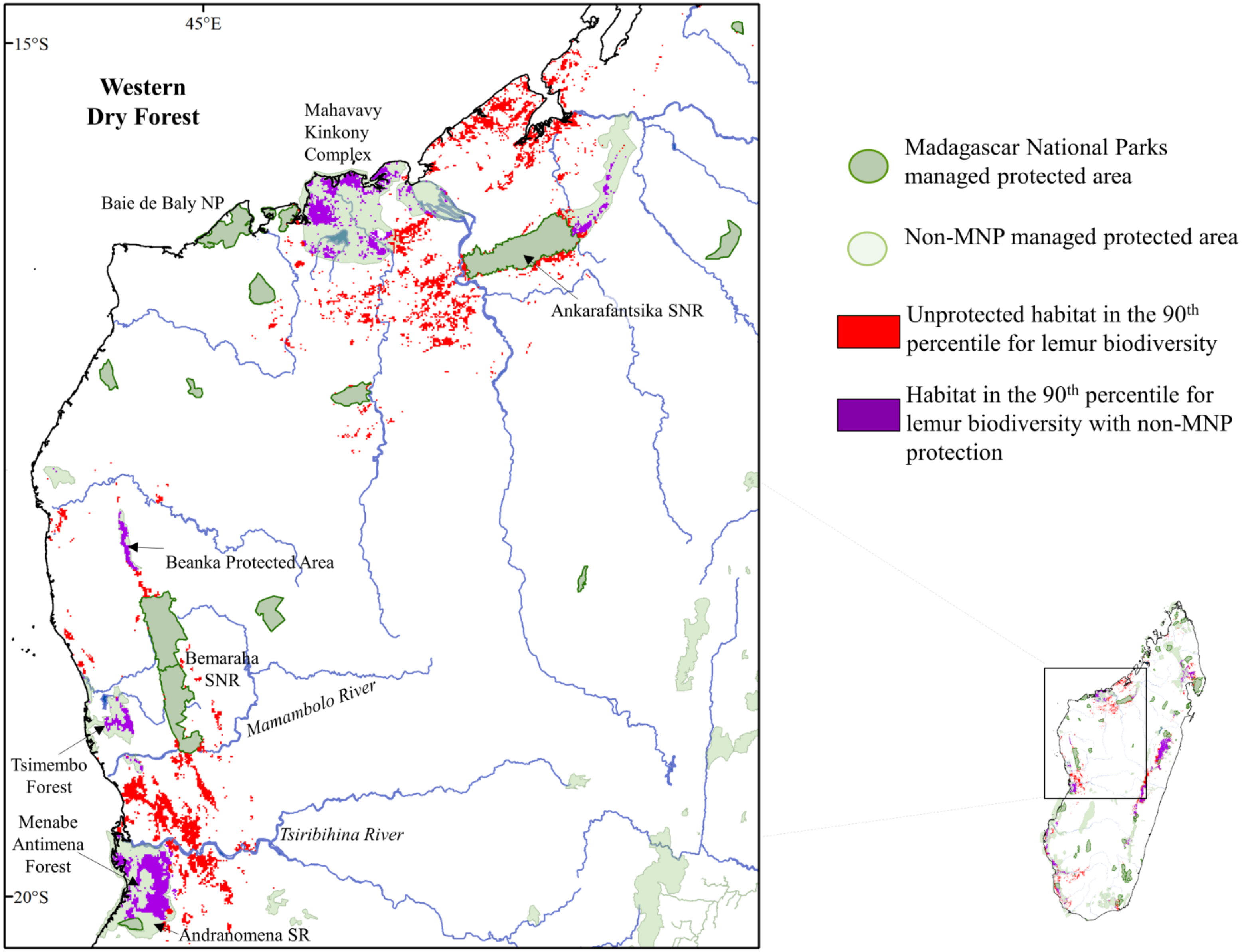


Figure 3.4. Areas suitable to many lemur species in Madagascar's western dry forests with low or no levels of protection.

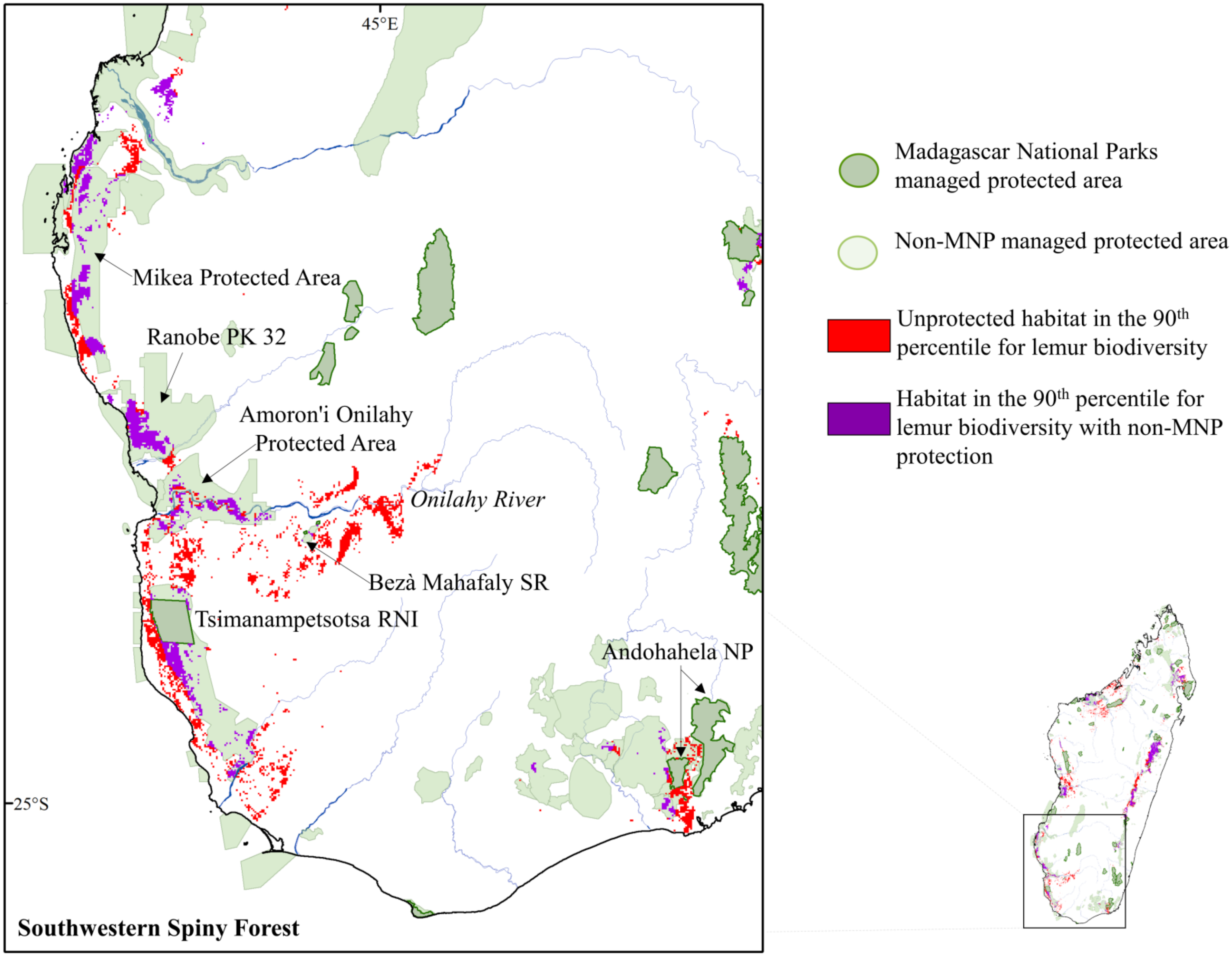


Figure 3.5. Areas suitable to many lemur species in Madagascar's southwestern spiny forests with low or no levels of protection.

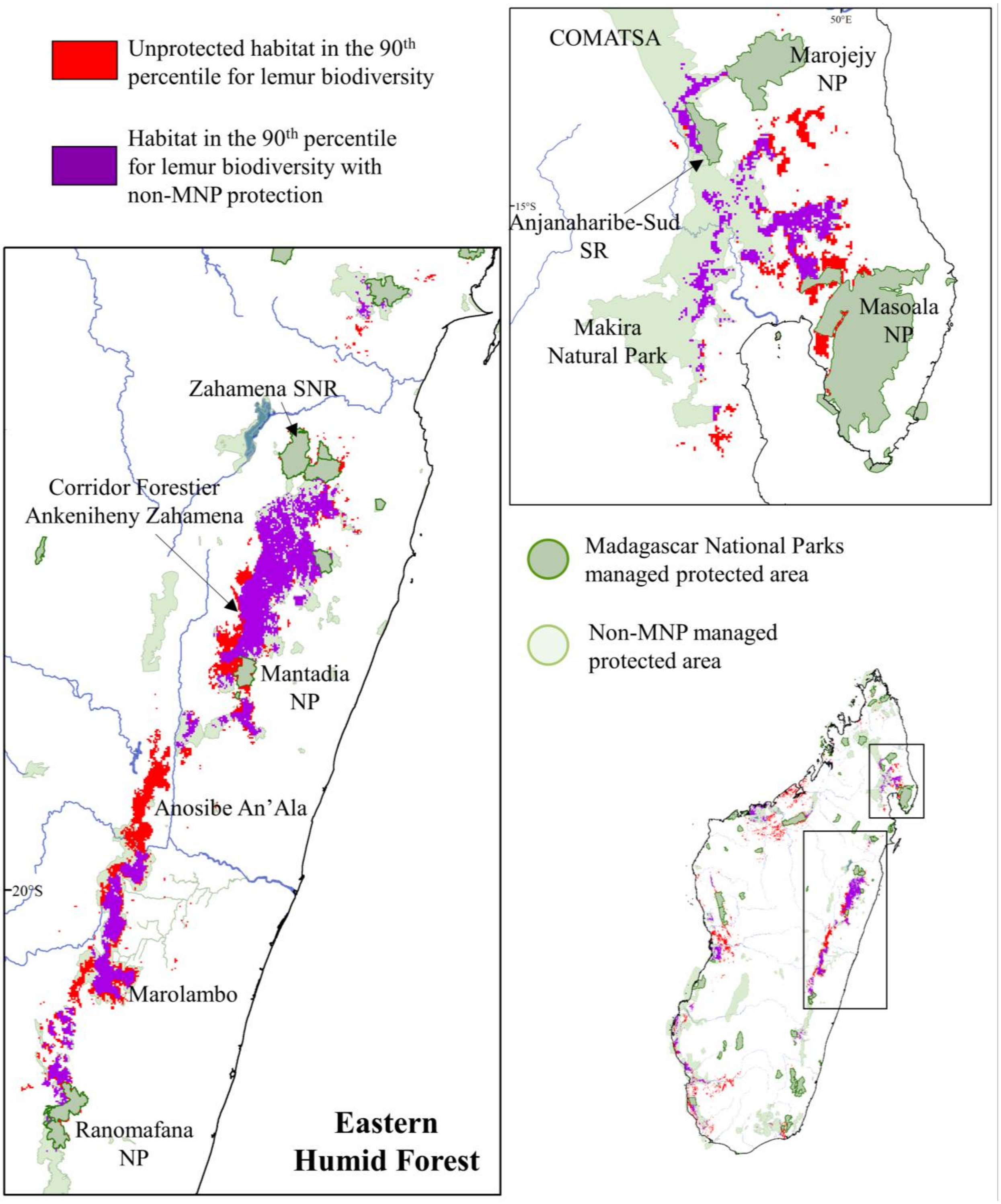


Figure 3.6. Areas suitable to many lemur species in Madagascar's eastern rainforests with low or no levels of protection.

Chapter 4: Ecological niche diversity in the sportive lemurs (genus *Lepilemur*)

Abstract

Primates, and lemurs especially, have undergone a taxonomic expansion in recent years due in large part to ever-increasing amounts of genetic data. One of the most hotly debated groups is the sportive lemurs (*Lepilemur* spp.), which has increased from eight species to 25 in the last two decades. Large rivers acting as dispersal barriers are thought to explain much, but not all, of these newly identified species' distributions. To investigate the role of local adaptation in this diversity, I constructed ecological niche models for 24 species of sportive lemur. I then compared sister species' niches to see if they evolved neutrally after a vicariant event or if they exhibited signals of selection. The genus as a whole did not exhibit Brownian motion in the evolution of their niches. Most sister taxa had significantly divergent ecological niches given the climatic background available to them, including the only sympatric species in the genus *L. ankaranensis* and *L.milanoii*. Other sister pairs experienced phylogenetic niche conservatism. Only one sister species pairing out of seven potentially exhibited neutral evolution in their ecological niches. Geographical neighbors were also largely diverged from each other. Rather than stochastic changes after vicariant events, many sportive lemurs seem to have undergone local adaptation. These changes may have accelerated and reinforced the allopatric speciation process initiated by riverine barriers. These unique ecological niches represent another line of evidence supporting the remarkable diversity in this genus.

Key words

Ecological niche; niche divergence; niche conservatism; *Lepilemur*; Madagascar; species delimitation

Introduction

The number of known primate species has greatly expanded as ever-increasing amounts of genetic data become available, with dozens of cryptic species of lemur identified. The 1994 edition of Lemurs of Madagascar recognized 50 taxa, the 2006 edition 71, and just four years later the 2010 edition included a further 30 taxa, totaling 101 (Mittermeier et al. 1994, 2006, 2010). More species have been recognized since then, and much work is needed to understand these newly identified species and how this diversity arose (Tattersall 2013).

The sportive lemurs (*Lepilemur* spp.) are emblematic of this boom in described species (Tattersall, 2013). Nearly forty years ago, Tattersall (1982) considered the species to be monotypic with four subspecies. These subspecies were eventually elevated to full species status, and increased research effort on nocturnal lemurs revealed fourteen new species of sportive lemur in 2006 alone. These new species were initially identified mostly through genetic and morphological data (Andriaholinirina et al., 2006; Louis et al., 2006; Rabarivola et al., 2006). As of 2019, 25 species of sportive lemur are supported by mitogenomic data (Lei et al., 2017).

However, these species numbers have been the topic of much debate in the literature, with Tattersall (2007) cautioning that geography, pelage variation, and mitochondrial distance cannot be used to reliably distinguish between a local population and a species. These lines of evidence constitute much of the data available for species within this genus (e.g. Louis et al. 2006; Craul et al. 2007), thus Tattersall (2007) finding firm evidence for only about a third of the

24 species at the time of writing. Markolf et al. (2011) argued that in order to use genetic distance and Population Aggregation Analysis to delimit species with the mitochondrial control region, at least ten samples per population are needed to conduct an adequate analysis of intraspecific variation. The difficulty of capturing primates in previously unsampled forests without established field sites can make this target sample size prohibitive.

Even so, one aspect of sportive lemur species that is not in debate is the influence large rivers have had on their biogeography. Most of the 25 species are delimited by major rivers, indicating that rivers acted as strong barriers, leading to allopatric speciation (Craul et al., 2007; Lei et al., 2017; Louis et al., 2006). However, rivers do not fully explain the distribution of sportive lemurs: two sister taxa in the north are partially sympatric (*L. ankaranensis* and *L. milanoii*), and *L. seali* in the northeast occurs on either side of the Antainambalana River (Craul et al., 2008; Louis et al., 2006).

Other hypotheses have been proposed to explain Madagascar’s the distribution of remarkable biodiversity, including one rooted in current climate (Pearson and Raxworthy, 2009) and the watershed or retreat-dispersion hypothesis (Wilmé et al., 2006). The latter argues that many Malagasy species’ current distributions could be explained by drier past climate causing forest to contract to higher elevation headwaters, isolating populations in lower elevation refugia, and ultimately resulting in speciation. However, no one model adequately explains the biodiversity patterns seen in lemurs (Yoder and Heckman, 2006; Pearson and Raxworthy, 2009). Considering current climatic niches alongside geographic distributions could help illuminate the extent to which current and past climates have fostered diversity in the genus *Lepilemur* (Kamilar and Muldoon, 2010; Pearson and Raxworthy, 2009). Ecological niche modeling can also be used to support species delimitation by quantitatively addressing questions about ecological uniqueness or exchangeability between closely related species (Crandall et al., 2000; Raxworthy et al., 2007; Rissler and Apodaca, 2007; Warren et al. 2008).

Lei et al. (2017) began these efforts by examining sportive lemur distributions in the context of these hypotheses using SEEVA analysis, which compares phylogenetic distance to differences in individual climate variables at occurrence records across sister species (Struwe et al., 2011). They found limited support for both the current climate and watershed hypotheses, but not in the north where unexplained sympatry occurs (Lei et al., 2017). However, SEEVA does not account for the differences between the climate available to species, despite the spatially autocorrelated nature of variables like precipitation and temperature (Struwe et al., 2011). Accessible climate may also differ significantly for allopatric species with highly limited ranges or for those occurring on a latitudinal gradient, both of which are true of sportive lemurs (Pearson and Raxworthy, 2009; Warren et al., 2008).

In this study, I further Lei et al.'s (2017) analyses by using Warren et al.'s (2008) background test on an expanded set of occurrence records and climate variables. The background test is suitable for allopatric species because it determines whether two species occupy more similar niches than would be expected given the climate available to them. As a null hypothesis, I assumed that sister species and geographic neighbors are separated by riverine barriers only and that they would exhibit phylogenetic niche conservatism, or the tendency to maintain ecological characteristics in the absence of strong selective pressures (Wiens and Graham, 2005). I also investigate whether phylogenetic distance across the genus correlates with ecological distance with a null hypothesis of changes in niches reflect Brownian or random motion over time.

Methods

A total of 885 reliable occurrence records were obtained from online databases, collaborators, and 85 peer-reviewed or gray literature papers (Supplementary Table 4.1). Localities without a known date that plotted in the occurrence area of another species were revised to match the taxonomy presented by Lei et al. (2017) or removed prior to analyses if near a poorly documented boundary between species. *Lepilemur mittermeieri* was treated as a junior synonym of *L. dorsalis* following Lei et al. (2017), and Ampasindava lemurs were considered to be *L. dorsalis*. Points were thinned as described in Chapter 3, resulting in 407 records across 25 species, with between 5 and 53 records each (Supplementary Table 4.1). Pseudo-absence points were drawn from a 50 km buffer drawn around all occurrence points for each species (Phillips et al., 2009).

Lepilemur spp. are entirely folivorous (Wright 1999); therefore, climate variables related to temperature, precipitation, and solar radiation, and soil were included in analyses (Table 4.1). They represent a 30-year window of very recent climate, centered on 1985, and were downloaded from the WorldClim, CliMond, SoilGrids, and WorldGrids databases (Hijmans et al., 2005; Kriticos et al., 2014). Only variables with a Pearson's r correlation coefficient < |0.85| were included in analyses (Merow et al., 2013). I constructed niche models in Maxent 3.4.1 (Phillips et al., 2017).

Model parameters were selected for each species using ENMeval 0.2.2 (Muscarella et al., 2014). Because many species had a small sample size after thinning, I chose from models with a ΔAIC ≤ 2, a method that improves models built with few occurrence records (Galante et al., 2014). Of those, I selected the parameters that had the mean tenth percentile omission rate closest to 0.1 to limit over- and under-fitting, and then the highest mean AUC (Warren and

Seifert, 2011; Muscarella et al., 2014). Model parameters and quality metrics are available in Supplementary Table 4.1. Models were then projected from this background to all of Madagascar. I assigned locations with climate outside the range of the training background a suitability score of zero (Elith et al., 2011).

All pairings of species' models were compared with two metrics of niche overlap, Schoener's D and Hellinger's I, calculated in ENMTools 1.4.4 (Warren et al. 2010; Tables 2 and 3). To test whether these values of niche overlap correlated with phylogenetic distance, I conducted two Mantel tests employing Pearson's correlation for 1,000 permutations in R 3.6.1 using the vegan package v 2.5-5 (Dixon, 2003; Cattin et al., 2004; Knouft et al., 2006; Warren et al., 2008). I used times to most recent common ancestor (TMRCA) from Lei et al. (2017) for phylogenetic distance. Schoener's D and Hellinger's I values were all subtracted from 1 prior to analyses to change the metrics from overlap scores to distances (from similarity to dissimilarity). In one Mantel test I compared Schoener's D values to TMRCA; in the other I compared Hellinger's I values to TMRCA.

Next, I investigated whether these metrics of niche overlap represented more niche conservatism or divergence than expected based on the climate available to species using Warren et al.'s (2008) background test. The background test does not assume that both species are drawn from the same geographic distribution and is well-suited to examining non-overlapping distributions (Warren et al., 2008). Niche models based on points drawn at random from the area available to Species A are repeatedly constructed to form a null distribution. These models are evaluated for niche similarity (Schoener's *D* and Hellinger's *I*) against the actual niche model of Species B.

If the niche similarity metric for Species A and B is greater than the distribution generated by the background test, the species are said to be more conserved than expected given the climate available to them. If the actual metric is lower, the species' niches have diverged. The test is run both directions (i.e. comparing species A to background points from species B's range and comparing B to background points from A), generating a two-tailed p-value. If both iterations of the test agree, confidence may be had in the assessment of niche conservatism or divergence (Warren et al., 2008).

I ran background tests for each pair of sister-species in the data set, as defined in Lei et al. (2017), and for geographic neighbors. The Maxent regularization multiplier, a value that accounts for the fittedness of the model, was set to the average of both species' most suitable values obtained in ENMeval (Supplementary Table 4.1). Each test was run for 100 iterations with 5000 background points sampled in ENMTools to obtain an approximate p-value resolution of 0.01. Two-tailed p-values of ≥ 0.975 indicated significant convergence, while values ≤ 0.025 indicated divergence. A majority of results (i.e. 3 out of 4 background tests across both D and I metrics) had to concur for a conclusion regarding the relationship of species' niches.

Results

Lepilemur spp. ecological niche models are displayed in Fig. 4.1. Most models predicted the occurrence data quite well, with average test AUC values of 0.82 (values range from 0-1, with models above 0.5 predicting better than chance; Table 1). The model for *L. jamesorum* had an AUC value of 0.5 and was excluded from analyses. For the remaining 24 species, Hellinger's I values ranged from 0.00-0.78, and Schoener's D values ranged from 0.00-0.96 (Supplementary Tables 4.2, 4.3). These metrics of niche overlap were used in the following tests.

Phylogenetic distance did not correlate with ecological niche dissimilarity for either Schoener's D or Hellinger's I, indicating that the genus as a whole does not exhibit Brownian motion in the evolution of its ecological niches (D: r = 0.01, p = 0.40; I: r = 0.03, p = 0.32). At the level of sister species, four pairs had significantly diverged niches, two had significantly conserved niches, and one pair had a result which indicated neither conservation nor divergence. The niches of the only species to occur in sympatry, *L. ankaranensis-L. milanoii*, have diverged significantly relative to the habitat available to them (Table 4.2; Fig 4.2). Three sister pairs are parapatric: *L. leucopus-L. petteri*, *L. hubbardorum-L. ruficaudatus,* and *L. hollandorum-L. seali* (Fig. 4.1). *L. leucopus-L. petteri* were significantly diverged; *L. hubbardorum-L. ruficaudatus* were significantly conserved (Fig. 4.3). *L. hollandorum-L. seali* were more conserved in one direction and more diverged in the other, indicating neither process describes these species exactly.

Sister species were not more diverged or conserved than geographic neighbors ($\chi^2 = 0.66$, p = 0.72). Geographic neighbors also exhibited a diversity of relationships, outlined in Table 4.3. Five species had significantly conserved niches, twelve had significantly diverged, and seven had inconclusive or conflicting results. Most species of sportive lemur occurring in the western dry forest were significantly diverged from their nearest geographic neighbors (8 out of 11 pairs), compared to only three out of nine eastern rainforest pairings.

Discussion

Riverine barriers have produced high levels of allopatric speciation across the sportive lemur genus (Craul et al., 2007; Louis et al., 2006), but many species' ecological niches evolved non-neutrally as well. Sportive lemur niches did not follow a pattern of Brownian motion as the genus diversified. Instead, sister species exhibit signatures of niche divergence and conservatism (Table 4.2). Thus, environmental conditions acted as selective pressures on sportive lemur populations during and/or after speciation events. These results underscore the uniqueness of these species and their utility as discrete evolutionarily significant units for conservation measures (Crandall et al., 2000).

Four pairs of sister species had significantly diverged niches, indicating that climatic conditions act as a barrier to gene flow for these lemurs, in addition to rivers (Wiens and Graham, 2005). One of these sister pairings, *L. ankaranensis-L. milanoii*, is sympatric in the Andrafiamena forest, the only known instance of sympatry in the genus *Lepilemur* (Fig. 4.2; Louis et al., 2006; Salmona et al., 2014). The limited ecological overlap between these species may have prevented adaptive gene flow between populations, resulting in speciation through diversifying selection (Orr and Smith, 1998; Wiens and Graham, 2005).

Even for the species limited by a riverine barrier, climatic niche evolution may have accelerated the allopatric speciation process (Desjardins-Proulx and Gravel, 2011). Local adaptation can reduce populations' ability to disperse through novel climate to other areas with suitable climate, reinforcing physical barriers (Kozak and Wiens, 2007, 2010). When niches are phylogenetically conserved (i.e. selected to stay the same over time), as in the case of *L. aeeclis* and *L. randrianasoloi*, species may be separated by a zone of unsuitable habitat that prevents ecologically similar species from coming into contact (Cooper and Freckleton, 2010; Wiens and Graham, 2005; Fig. 4.3).

When niches are diverged, geographically close species that may be able to cross a riverine barrier cannot colonize the different climate on the other side. This is apparently true of many geographic neighbors in the genus *Lepilemur* (Table 4.3). Most neighbors tend to have significantly diverged niches, indicating that they are ecologically non-exchangeable (Crandall et al., 2000). This trend was more present in the western dry forests than the eastern humid forests of Madagascar. Although additional Malagasy taxa should be studied before drawing conclusions, this finding tentatively supports those of Kozak and Wiens (2010). They observed that niche diversification tends to be greater in places with fewer taxa. Madagascar's eastern rainforests have greater species richness that its western dry forests (Vences et al., 2009).

Due to its heterogenous landscapes and complex climatic past, Pearson and Raxworthy (2009) cautioned against a "one size fits all" explanation for Madagascar's remarkable endemism. This advice seems to hold for the sportive lemurs, despite the apparent influence of rivers on their biogeography. Instead of the Brownian motion that might be expected from purely allopatric speciation, *Lepilemur* climatic niches exhibit strong signals of divergence and conservatism. Local adaptation may have intensified the splits introduced by riverine barriers, resulting in the genetic diversity observed today (Kozak and Wiens, 2007, 2010; Lei et al., 2017). The ecological non-exchangeability of closely related taxa also represents another line of evidence for species within this clade (Crandall et al., 2000; Raxworthy et al., 2007; Rissler and Apodaca, 2007). Rather than neutrally-evolving lineages, sportive lemurs may represent one of the widest spread adaptive radiations of Madagascar (Rundell and Price, 2009; Wilmé et al., 2006).

Table 4.1. Environmental variables included in niche model construction.

Climate Variable	**Source**
BIO2: Mean diurnal temperature range	WorldClim v1.4; (Hijmans et al., 2005)
BIO3: Isothermality	WorldClim v1.4; (Hijmans et al., 2005)
BIO4: Temperature seasonality	WorldClim v1.4; (Hijmans et al., 2005)
BIO5: Max temperature of warmest month	WorldClim v1.4; (Hijmans et al., 2005)
BIO6: Min temperature of coldest month	WorldClim v1.4; (Hijmans et al., 2005)
BIO12: Annual Precipitation	WorldClim v1.4; (Hijmans et al., 2005)
BIO13: Precipitation of Wettest Month	WorldClim v1.4; (Hijmans et al., 2005)
BIO16 Precipitation of wettest quarter	WorldClim v1.4; (Hijmans et al., 2005)
BIO17: Precipitation of driest quarter	WorldClim v1.4; (Hijmans et al., 2005)
BIO18: Precipitation of warmest quarter	WorldClim v1.4; (Hijmans et al., 2005)
BIO21: Highest weekly solar radiation	CliMond v1.2; (Kriticos et al., 2014)
BIO22: Lowest weekly solar radiation	CliMond v1.2; (Kriticos et al., 2014)
BIO24: Radiation of wettest quarter	CliMond v1.2; (Kriticos et al., 2014)
BIO25: Radiation of driest quarter	CliMond v1.2; (Kriticos et al., 2014)
Slope	WorldGrids; (Becker et al., 2009; Amante and Eakins, 2009)
Topographic wetness	WorldGrids; (Becker et al., 2009; Amante and Eakins, 2009)
Depth to bedrock	SoilGrids v0.5; (Hengl et al., 2017)
Soil acidity (pH in H_2O)	SoilGrids v0.5; (Hengl et al., 2017)
Soil organic carbon content	SoilGrids v0.5; (Hengl et al., 2017)

Table 4.2. Approximate p-values generated by Warren et al.'s (2008) background test of niche similarity for sister *Lepilemur* species. Conserved niches are more similar than expected based on the climatic background available to species, while diverged niches are less similar.

Sister species		**Comparing Species A to Species B's background**		**Comparing Species B to Species A's background**		**Result**
Species A	Species B	I	D	I	D	
L. aeeclis	*L. randrianasoloi*	> 0.99	< 0.01	< 0.01	< 0.01	conserved
L. ahmansonorum	*L. sahamalazensis*	> 0.99	> 0.99	> 0.99	> 0.99	diverged
L. ankaranensis	*L. milanoii*	> 0.99	> 0.99	> 0.99	> 0.99	diverged
L. edwardsi	*L. grewcockorum*	> 0.99	> 0.99	> 0.99	> 0.99	diverged
L. hollandorum	*L. seali*	< 0.01	< 0.01	> 0.99	> 0.99	inconclusive
L. hubbardorum	*L. ruficaudatus*	< 0.01	< 0.01	> 0.99	< 0.01	conserved
L. leucopus	*L. petteri*	> 0.99	> 0.99	> 0.99	> 0.99	diverged

Table 4.3. Approximate p-values generated by Warren et al.'s (2008) background test of niche similarity for geographic neighbors in the *Lepilemur* genus. Conserved niches are more similar than expected based on the climatic background available to species, while diverged niches are less similar.

Geographic Neighbors		**Comparing Species A to Species B's background**		**Comparing Species B to Species A's background**		**Result**
Species A	Species B	I	D	I	D	
L. aeeclis	*L. ahmansonorum*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. aeeclis	*L. edwardsi*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. ahmansonorum	*L. randrianasoloi*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. ankaranensis	*L. dorsalis*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. ankaranensis	*L. milanoii*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. ankaranensis	*L. septentrionalis*	< 0.01	< 0.01	< 0.01	> 0.99	diverged
L. betsileo	*L. microdon*	< 0.01	0.74	< 0.01	< 0.01	diverged
L. betsileo	*L. mustelinus*	> 0.99	> 0.99	< 0.01	< 0.01	inconclusive
L. betsileo	*L. milanoii*	< 0.01	< 0.01	> 0.99	< 0.01	diverged
L. dorsalis	*L. sahamalazensis*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. dorsalis	*L. seali*	> 0.99	> 0.99	> 0.99	> 0.99	conserved
L. edwardsi	*L. otto*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. fleuretae	*L. leucopus*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. fleuretae	*L. wrightae*	> 0.99	> 0.99	> 0.99	> 0.99	conserved
L. grewcockorum	*L. otto*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. grewcockorum	*L. sahamalazensis*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. hollandorum	*L. mustelinus*	> 0.99	> 0.99	< 0.01	< 0.01	inconclusive
L. hollandorum	*L. seali*	> 0.99	> 0.99	< 0.01	< 0.01	inconclusive
L. hubbardorum	*L. petteri*	> 0.99	> 0.99	> 0.99	> 0.99	conserved
L. hubbardorum	*L. ruficaudatus*	> 0.99	> 0.99	< 0.01	> 0.99	conserved
L. leucopus	*L. petteri*	< 0.01	< 0.01	< 0.01	< 0.01	diverged
L. microdon	*L. wrightae*	< 0.01	0.27	< 0.01	< 0.01	diverged
L. randrianasoloi	*L. ruficaudatus*	> 0.99	> 0.99	> 0.99	> 0.99	conserved
L. scottorum	*L. seali*	> 0.99	> 0.99	< 0.01	< 0.01	inconclusive

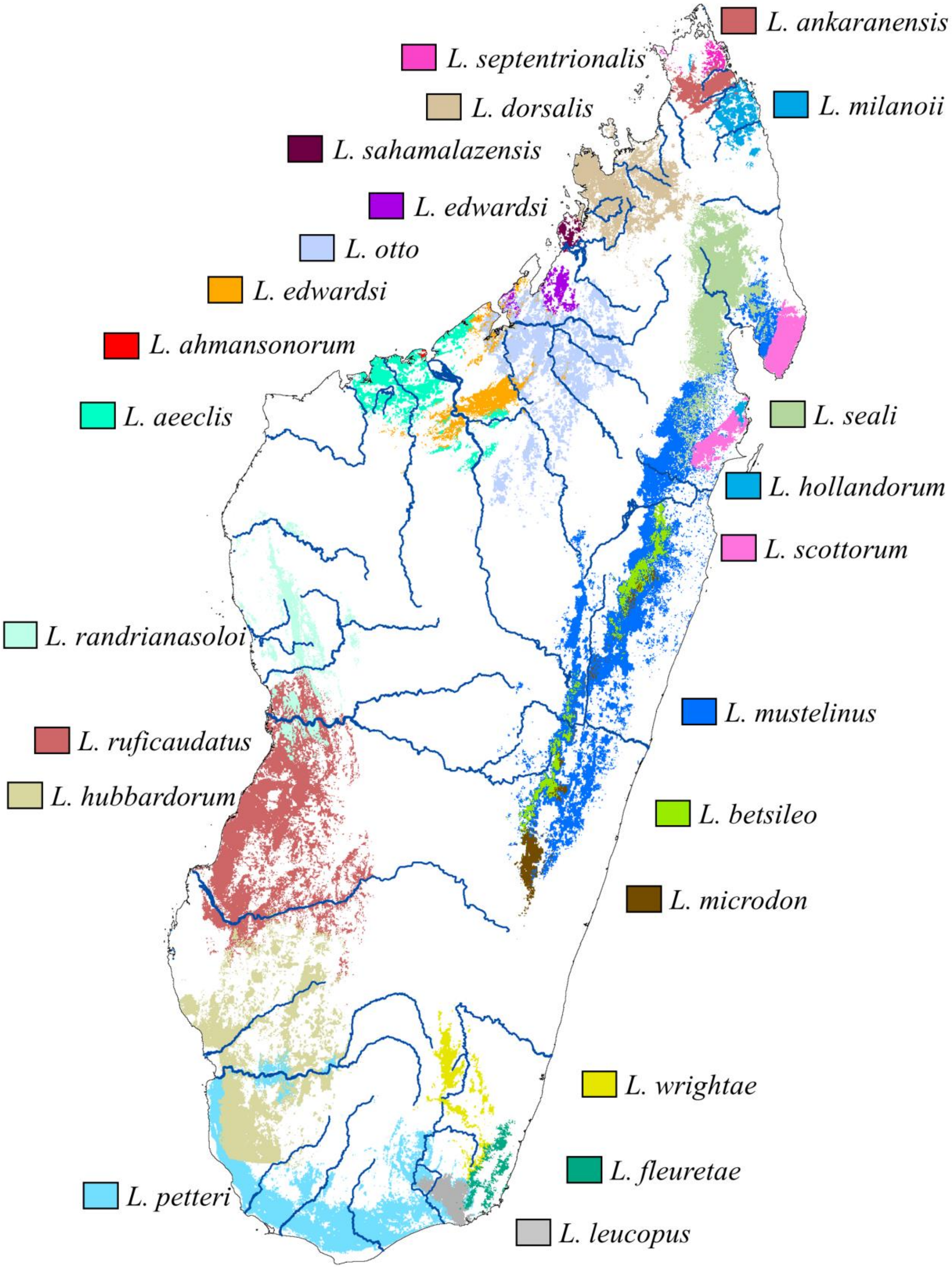


Figure 4.1. *Lepilemur* spp. ecological niche models binarized with a 10th percentile training presence threshold.

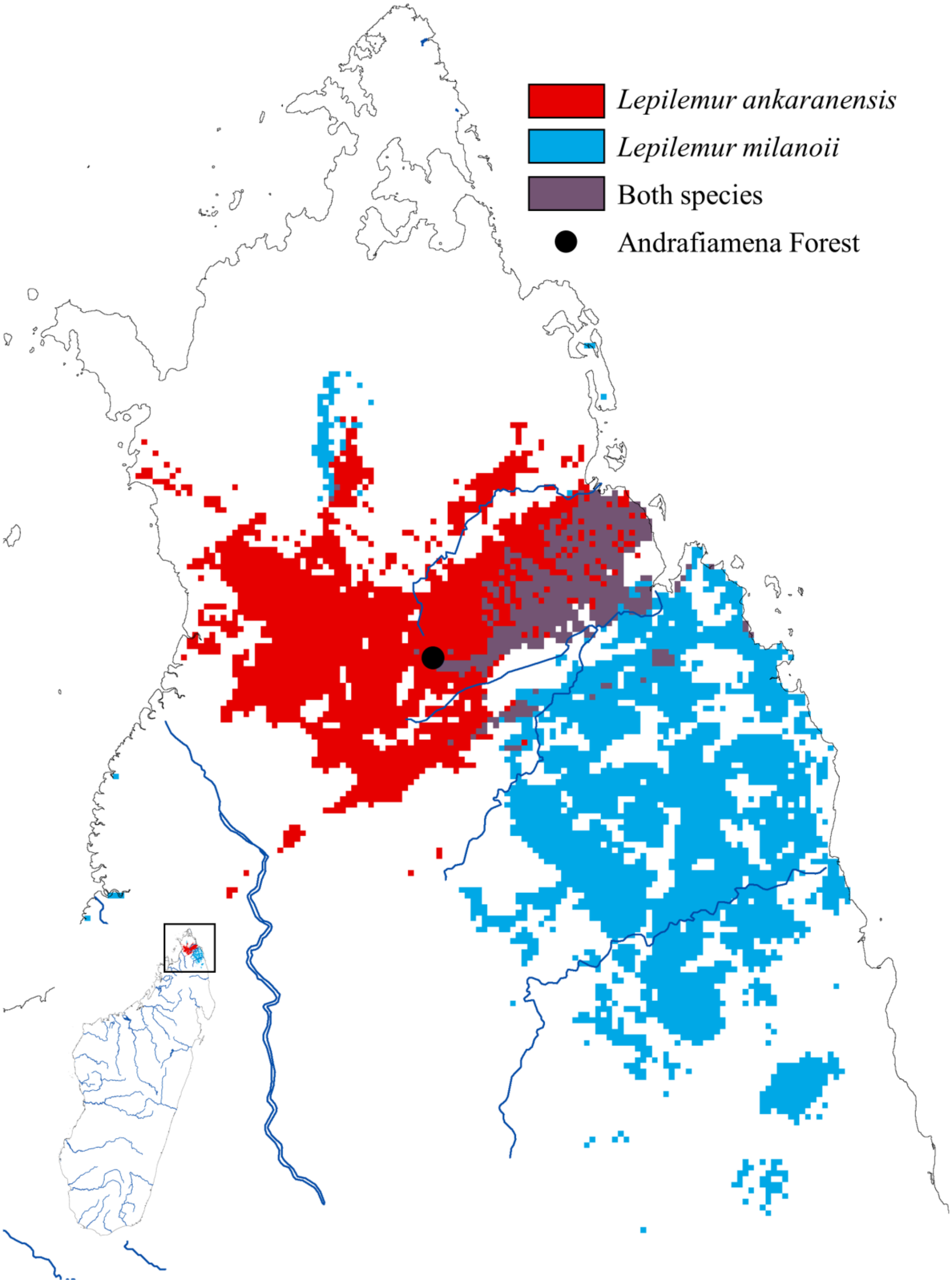


Figure 4.2. Thresholded ecological niche models for *Lepilemur ankaranensis* and *L. milanoii*. Andrafiamena Forest, the only known location of sympatry in *Lepilemur* spp. is marked.

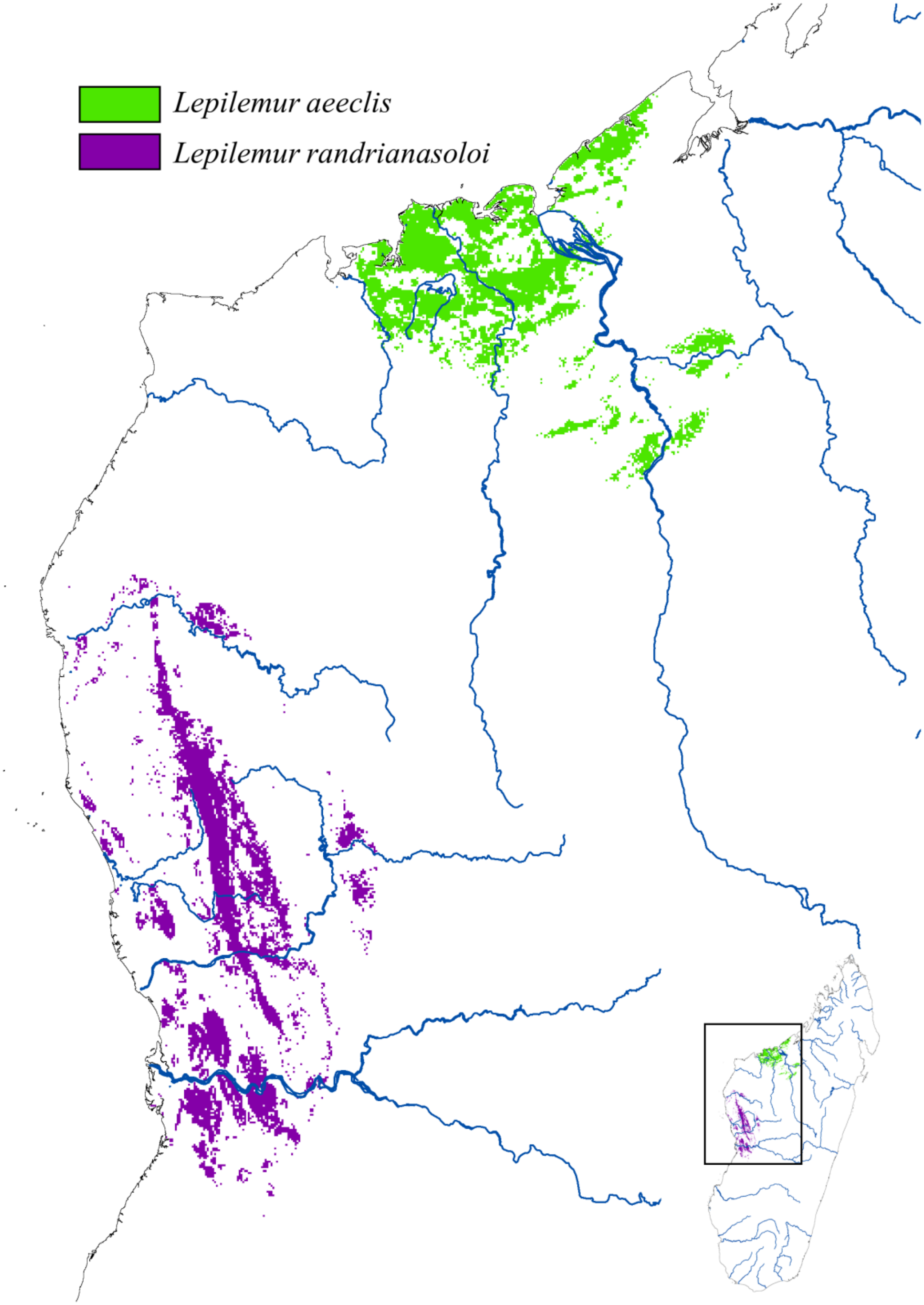


Figure 4.3. Thresholded ecological niche models for *Lepilemur aeeclis* and *L. randrianasoloi*. These species' niches are conserved, but a zone of unsuitable habitat separates them.

Chapter 5: Stationary rivers and shifting climate: how much suitable habitat will lemurs be able to access as Madagascar warms?

Abstract

Climate change threatens global biodiversity, with suitable habitat expected to shift or contract for many species. Assessing the severity of these changes is an increasingly necessary component of conservation planning. Projecting current ecological niches onto future climate scenarios can help identify priority areas for conservation, but these models require careful interpretation to be of use for dispersal-limited species. Here, I examine how riverine barriers alter future habitat predictions for a group of highly endemic primates, the sportive lemurs (*Lepilemur* sp.). When dispersal ability is ignored, climate change does not appear to have much of an effect at the genus level by the 2070s: some species' habitat is lost, but other species expand into those areas. When future projections are limited to accessible areas, many of those gains vanish. Sportive lemurs lose on average 25% of their current habitat, and five species are predicted to have no suitable, accessible habitat by 2070. Further, all habitat available in 2070 will represent novel climatic conditions relative to the niches they currently occupy. I discuss the likelihood that sportive lemurs will persist under novel climate conditions. These results demonstrate that species' abilities to track habitat as climate change progresses can greatly affect projections of future habitat and that species-specific knowledge is critical to interpreting ecological niche projections for conservation planning.

Key words: climate change vulnerability; ecological niche modeling; sportive lemurs; *Lepilemur*; Madagascar; riverine barriers

Introduction

Assessing species vulnerability to climate change is an increasingly important component of conservation plans (Foden et al., 2013, 2019; Pacifici et al., 2015; Thomas et al., 2004). Climate change drives demographic declines as large as those caused by habitat loss and its impact is only expected to increase over time (Selwood et al., 2015). Correlative models of ecological niches are one method for evaluating the impact of climate change, and they can be particularly useful for species like primates where physiological tolerances can be difficult to observe directly (Anderson, 2013; Graham et al., 2016). By projecting species' current climatic envelope onto projected future climate, we can predict changes in available habitat and identify species at risk for extinction due to climate change (Elith et al., 2011; Foden et al., 2019; Weins et al., 2009).

However, climate-based models need to be evaluated in the context of other risks to inform conservation efforts (Pearson and Dawson, 2003). Habitat loss and fragmentation are threats on their own and are often exacerbated by warming temperatures (Mantyka-Pringle et al., 2012). Biotic factors, like climate-driven changes in food availability and novel contact with competitors, can render climatically suitable habitat uninhabitable (Eronen et al., 2017; HilleRisLambers et al., 2013). Critically, species must be able to disperse to future, suitable habitat if it does not occupy the same geographic space as their current distribution (Schloss et al., 2012).

Dispersal ability is often highly species-specific and can depend on landscape features occurring at smaller scales than ecological niche models (Anderson, 2013; Pearson and Dawson, 2003). Several global-scale assessments have chosen between two dispersal options: unlimited and none at all, but acknowledge that more realistic abilities fall in between those extremes

(Graham et al., 2016; Thomas et al., 2015). Others have calculated a maximum rate of dispersal based on body mass and other traits (Schloss et al., 2012). For the lemurs of Madagascar, Brown and Yoder (2015) limited dispersal to 1.4 km/year but assumed no physical barriers. While species' limitations may not change broad trends for continent or ecosystem-wide analyses, physical barriers can matter a great deal to individual species.

In fact, riverine barriers are responsible for much of Madagascar's biodiversity (Goodman and Ganzhorn, 2004; Pearson and Raxworthy, 2009; Wilmé et al., 2006). Perhaps no group demonstrates the influence of rivers limiting dispersal more clearly than the sportive lemurs (*Lepilemur* sp.). The genus is widely distributed across Madagascar, but sympatry has only been documented in a single forest (Louis et al., 2006). Most of the 25 species are delimited from each other by large rivers (Andriaholinirina et al., 2006; Craul et al., 2007; Lei et al., 2017; Louis et al., 2006). The majority of these speciation events took place in the Plio-Pleistocene, indicating that these rivers have persisted over time and past climate changes (Lei et al., 2017).

In this chapter, I use sportive lemurs and their sensitivity to rivers to investigate how physical barriers affect accessible habitat as climate change progresses. I also consider how much of their future range depends on adapting to novel climate. Niche modeling algorithms often extrapolate when asked to project onto one or more climate variables outside of the training range. Elith et al. (2011) recommend "extreme care" when drawing inferences from these projections of suitability in novel climates. I hypothesize that when physical barriers to dispersal and novel climate are considered, sportive lemurs will have access to less habitat than when barriers are excluded from climate change vulnerability assessments.

Methods

A total of 885 reliable occurrence records were obtained from online databases, collaborators, and 85 peer-reviewed or gray literature papers (Supplementary Table 5.1). Localities without a known date that plotted in the occurrence area of another species were revised to match the taxonomy presented by Lei et al. (2017) or removed prior to analyses if near a poorly documented boundary between species. *Lepilemur mittermeieri* was treated as a junior synonym of *L. dorsalis* following Lei et al. (2017), and Ampasindava lemurs were considered to be *L. dorsalis*. Localities were thinned as described in Chapter 3, resulting in 407 records across 25 species, with between 5 and 53 records each (Supplementary Table 5.1).

Nine climate variables were downloaded from the WorldClim database at 30 arc-second (~1 km) resolution for "current" climate (centered on 1985), and two future time points, centered on 2050 and 2070 (Hijmans *et al.*, 2005; Table 5.1). Only climate variables with Pearson's r correlation coefficients $\leq |0.85|$ were included in analyses (Merow *et al.*, 2013). I used future climate projections generated by the Community Climate System Model v. 4 and selected a middling climate change scenario, Representative Concentration Pathway 4.5, for analyses. I excluded elevation as a variable in favor of other highly correlated variables that will change over time (e.g. temperature, precipitation). Model parameter choice and construction followed methods outlined in Chapter 3 and model quality metrics are available in Supplementary Table 5.2. All models were binarized using the 10th percentile training presence as a threshold and areas of suitability were calculated for each time period using `rgeos` in R.

To investigate whether physical barriers could limit dispersal to future habitat by sportive lemurs, I generated inter-river polygons extending to known barriers for each species in ArcMap (Supplementary Fig. 5.1). I used these polygons to limit species' distribution models to only those areas that sportive lemurs could disperse to and recalculated areas of suitability. All polygons terminated at either a river, the coast, or in the unforested central highlands. When a river's status as a barrier to dispersal was uncertain due to a lack of fieldwork near the river, I assumed it could be crossed, that absence on the other side of the river might be a result of competitive exclusion, and chose the next known barrier river for the polygon (note that the polygons for *L. betsileo*, *L. jamesorum*, and *L. microdon* in Supplementary Fig. 5.1 are identical for this reason). This choice meant that estimates of accessible, suitable habitat in future scenarios err on the side of optimism. I also calculated how much predicted habitat in the future models fell within the current training range and how much relied on the suitability of novel climatic conditions.

Results

Assuming perfect dispersal ability across Madagascar and the ability to occupy novel climate, sportive lemurs lost very little habitat to climate change. As a genus, they lost 3% of their net suitable area to climate change by 2050 and 0% by 2070 (Table 5.2; Fig. 5.1.A). Responses were more variable for individual species, with nine species out of 25 not experiencing much change in the area of their habitat by 2070 (defined as maintaining between 75% and 125% of their current suitable area, following Brown and Yoder, 2015). Three species, *L. grewcockorum*, *L. microdon*, and *L. petteri*, actually expanded their ranges (beyond 125% of their current area; Fig. 5.2). Six species lost more than 90% of their suitable space by 2070, with two of these species losing all their habitat to climate change: *L. septentrionalis* and *L. ruficaudatus* (Table 5.3).

A more realistic dispersal scenario, in which large rivers are treated as barriers to sportive lemurs, revealed greater losses (Tables 5.2, 5.3; Fig. 5.1.B). Accessible, climatically suitable habitat represented roughly one-third of the areas obtained assuming perfect dispersal (Table 5.4). When barriers were considered, climate change resulted in losses of 21% of accessible habitat by 2050 and 24% by 2070 on average (Table 5.2). Eight species experienced little change in their accessible habitat due to climate change by 2070. *L. microdon* and *L. petteri* still gained suitable habitat even when riverine barriers were considered (Fig. 5.3), but *L. grewcockorum* actually lost 97% of its accessible habitat (Table 5.3; Fig. 5.2). The other 15 species experienced losses, and *L. edwardsi*, *L. leucopus* and *L. tymerlachsoni* joined *L. septentrionalis* and *L. ruficaudatus* in disappearing entirely (Table 5.3).

When Maxent is constrained from predicting suitable habitat in novel climatic conditions, much smaller areas are suitable in the future. Only 53% of their current climate habitat is available to sportive lemur species on average in 2050. By 2070, 100% of available habitat would include novel climatic conditions outside the current realized niches of these lemurs.

Discussion

Barriers to dispersal greatly reduce the amount of suitable space sportive lemurs will be able to access as climate change progresses (Fig. 5.1). Models assuming perfect dispersal did tend to reflect the general trends of gains or losses of the river-limited models. However, sportive lemurs lost proportionally more of their river-limited habitat to climate change (Table 5.3). These losses included five species losing all accessible, climatically suitable space by 2070, even when novel climatic conditions were included. Three of those species would appear to maintain some suitable area when barriers are not considered, underscoring the point made by others that future projections assuming perfect dispersal can be unrealistically optimistic (Schloss et al., 2012; Thomas et al., 2004).

Remarkably, one species that appeared to gain suitable space under perfect dispersal, *L. grewcockorum*, lost nearly all of its accessible habitat to climate change (Fig. 5.2). This climatically suitable, but inaccessible, habitat occurs south of the Sofia River, which separates *L. grewcockorum* from its sister species *L. edwardsi* (Lei et al., 2017). *L. edwardsi* is projected to lose all of its accessible habitat by 2050 (Table 5.3). It is thus possible that instead of seeing an expansion of *L. grewcockorum*, the area between the Betsiboka and Sofia Rivers will lose sportive lemurs altogether in the next 30 years.

Nonetheless, species with projected total losses, like *L. edwardsi*, may not disappear outright. The models in this study are trained on the realized niches of sportive lemurs, not their fundamental niches. The fundamental niche represents the entirety of suitable conditions, whereas the realized niche represents the occupied subset thereof (Phillips and Dudik, 2008; Elith et al., 2011; Wiens et al., 2009). Even if the occurrence localities here represented complete sampling, the range of climatic conditions at sportive lemur occurrence records would still be limited by barriers and interspecific competition (Ganzhorn, 1997; Wright, 1999). Thus, species with very narrow geographic ranges like *L. septentrionalis* and *L. tymerlachsoni* may tolerate future climate. It is difficult to tell where those tolerable conditions might occur and whether they will be accessible without mechanistic studies, complicating conservation planning for these primates.

Indeed, we must hope that sportive lemurs are able to persist in novel climatic conditions. By 2070, nowhere in Madagascar will resemble the current climate conditions to which lemurs are adapted (Tables 5.2, 5.3). If sportive lemurs are not able to inhabit or rapidly adapt to novel conditions, they could go extinct within the century. Lemurs do have a suite of adaptations that contribute to their resilience in harsh environments, including the low basal metabolic rates, fibrous diets, and small group sizes exhibited by sportive lemurs (Wright, 1999, 2006). While these traits may help species temporarily endure climate change, it is unclear how much more desiccation and extreme weather events lemurs can adapt to and whether they can do so rapidly enough to outpace anthropogenic climate change (Dunham et al., 2011; Wright, 2006).

These adaptations to extreme climate are predicated on a complex network of biotic interactions and seasonal resources which may also be threatened by climate change (Anderson, 2017; Eronen et al., 2017). One of the basic resources required by sportive lemurs are holes in mature trees for sleeping (Rasoloharijaona et al., 2008). Unfortunately, forest loss is also an accelerating threat to lemurs, not only because of lost habitat but also increased contact with humans (Lehman et al., 2006a; Vieilledent et al., 2018). Fragmentation has left 46% of remaining forest within 100 m of the edge, close enough for the forest micro-climate to be altered (Broadbent et al., 2008; Vieilledent et al., 2018).

Accessible, forested areas that will maintain suitable climate over time should be prioritized for limited conservation resources. While corridors between currently suitable climate and future habitat areas will help more vagile species (Brown and Yoder, 2015), the inability of sportive lemurs and other species to cross large rivers limits their utility. The results of this study also demonstrate the importance of considering species-specific physical barriers to dispersal when modeling habitat under climate change. Conservation actions based on over-projection could be at best inefficient, and at worst waste time that lemurs do not have.

Table 5.1. Climate variables included in niche model construction.

Climate Variable	**Source**
BIO2: Mean diurnal temperature range	WorldClim v1.4; (Hijmans et al., 2005)
BIO3: Isothermality	WorldClim v1.4; (Hijmans et al., 2005)
BIO4: Temperature seasonality	WorldClim v1.4; (Hijmans et al., 2005)
BIO5: Max temperature of warmest month	WorldClim v1.4; (Hijmans et al., 2005)
BIO6: Min temperature of coldest month	WorldClim v1.4; (Hijmans et al., 2005)
BIO12: Annual Precipitation	WorldClim v1.4; (Hijmans et al., 2005)
BIO13: Precipitation of Wettest Month	WorldClim v1.4; (Hijmans et al., 2005)
BIO17: Precipitation of driest quarter	WorldClim v1.4; (Hijmans et al., 2005)
BIO18: Precipitation of warmest quarter	WorldClim v1.4; (Hijmans et al., 2005)

Table 5.2. Space suitable to at least one species of sportive lemur based on ecological niche models, with and without Maxent's extrapolation into novel climate, and with and without riverine barriers to dispersal.

Model limitations	Climatically suitable area (km^2)			Percentage of current suitable area lost	
	Current	2050	2070	by 2050	by 2070
Models including novel climate	553,357	539,308	553,729	3%	0%
River-limited models including novel climate	216,073	170,047	164,634	21%	24%
Models without novel climate	301,428	181,476	0	40%	100%
River-limited models without novel climate	188,075	102,070	0	46%	100%

Table 5.3 is available in an attached Excel file. Climatically suitable areas in km^2 as calculated by projecting current ecological niche models into future climate scenarios and then applying different dispersal rules.

Table 5.4. The percentage of projected models represented by inaccessible, but climatically suitable, areas when riverine barriers are ignored.

Species	Climatically suitable area lost to river barriers (with novel projections)			Climatically suitable area lost to river barriers (exclusive of novel projections)		
	Current	2050	2070	Current	2050	2070
Lepilemur aeeclis	98%	97%	98%	72%	89%	0%
Lepilemur ahmansonorum	99%	99%	99%	96%	100%	0%
Lepilemur ankaranensis	89%	85%	89%	0%	0%	0%
Lepilemur betsileo	32%	38%	48%	11%	0%	0%
Lepilemur dorsalis	78%	81%	82%	45%	70%	0%
Lepilemur edwardsi	65%	100%	95%	24%	100%	0%
Lepilemur fleuretae	89%	90%	90%	77%	83%	0%
Lepilemur grewcockorum	94%	92%	99.9%	88%	56%	0%
Lepilemur hollandorum	68%	67%	68%	57%	54%	0%
Lepilemur hubbardorum	41%	63%	46%	20%	35%	0%
Lepilemur jamesorum	75%	90%	80%	24%	55%	0%
Lepilemur leucopus	30%	24%	100%	1%	0%	0%
Lepilemur microdon	38%	31%	29%	29%	23%	0%
Lepilemur milanoii	25%	27%	20%	25%	27%	0%
Lepilemur mustelinus	87%	86%	86%	73%	68%	0%
Lepilemur otto	93%	93%	93%	81%	81%	0%
Lepilemur petteri	76%	77%	79%	17%	8%	0%
Lepilemur randrianasoloi	62%	96%	78%	12%	42%	0%
Lepilemur ruficaudatus	71%	73%	0%	14%	22%	0%
Lepilemur sahamalazensis	69%	48%	62%	55%	0%	0%
Lepilemur scottorum	86%	89%	87%	54%	76%	0%
Lepilemur seali	36%	29%	1%	20%	17%	0%
Lepilemur septentrionalis	2%	0%	0%	2%	0%	0%
Lepilemur tymerlachsoni	99.84%	99.99%	100%	68%	33%	0%
Lepilemur wrightae	95%	98%	98%	84%	95%	0%
MEAN	**68%**	**71%**	**69%**	**42%**	**45%**	**0%**

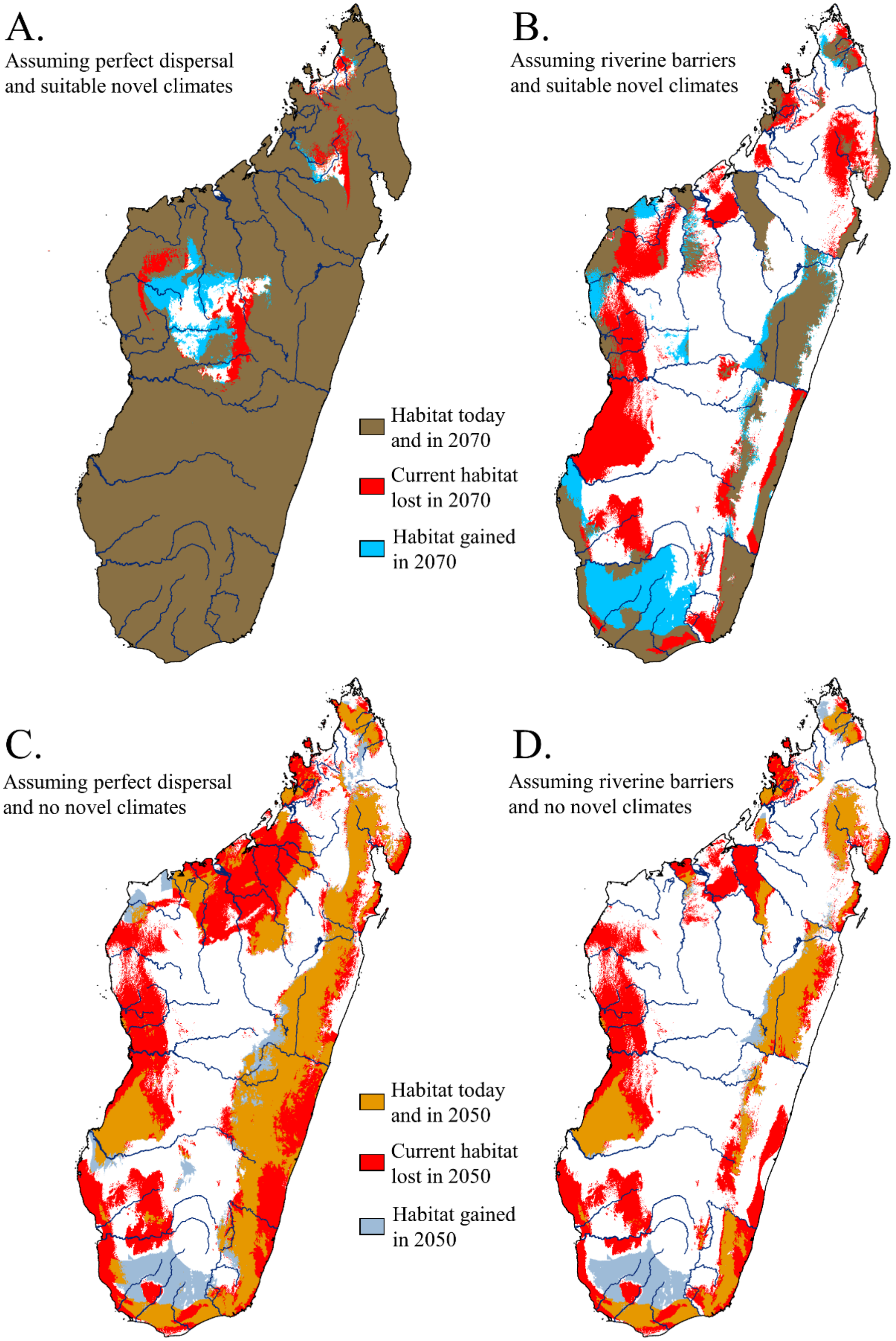


Figure 5.1. Areas that are climatically suitable for at least one species of sportive lemur now and in the future, assuming unlimited dispersal ability (A. and C.), and treating large rivers as barriers to dispersal (B. and D.). The models without novel climate (C. and D.) only include changes by 2050 because there was no non-novel climate in 2070 for sportive lemurs.

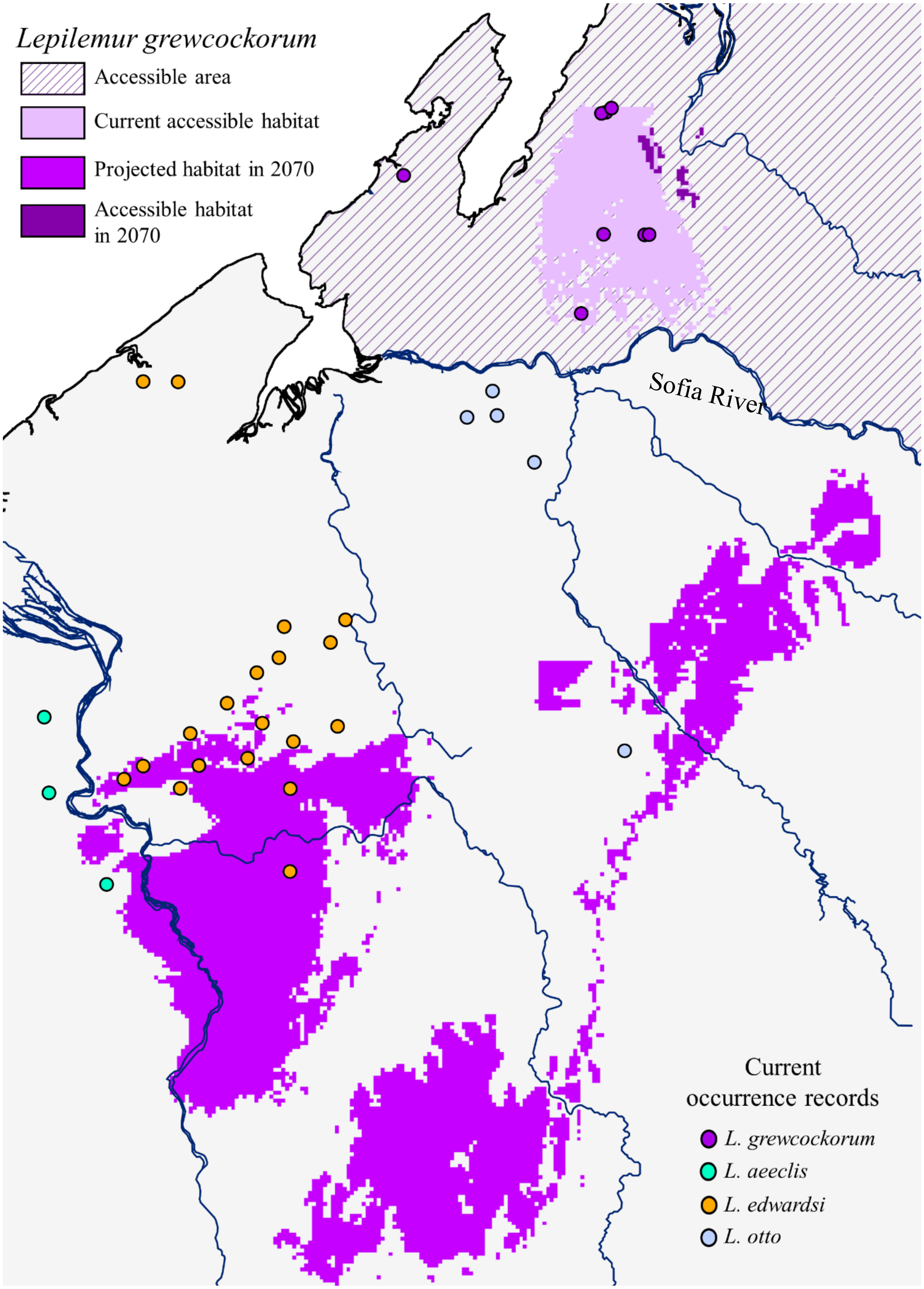


Figure 5.2. Northwestern Madagascar and thresholded models of suitable and accessible areas for *Lepilemur grewcockorum* reflecting current climate and projections for 2070. Suitable but inaccessible areas south of the Sofia River are depicted in bright pink.

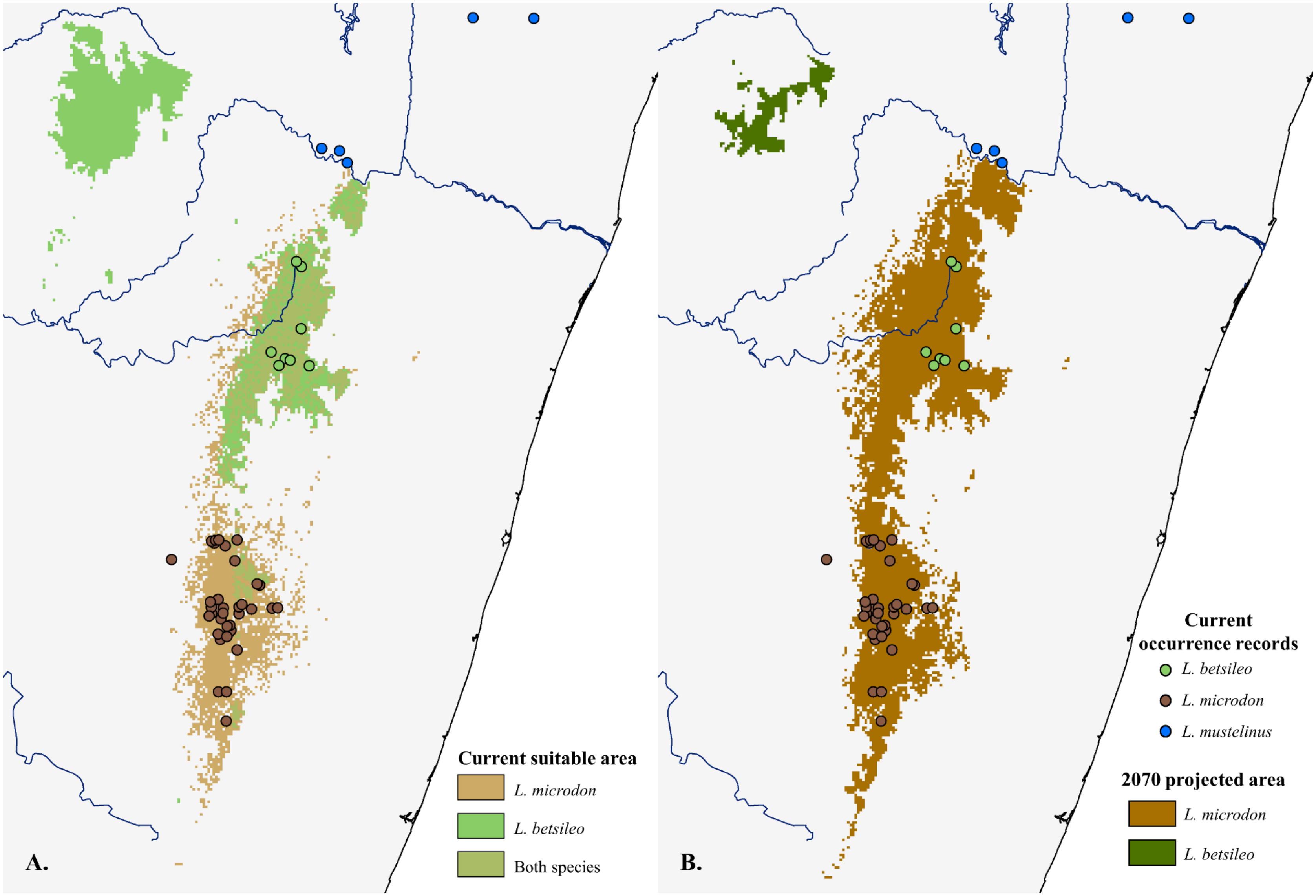


Figure 5.3. The east coast of Madagascar and A.) current climate models of suitable area for *Lepilemur microdon*, which is projected to increase as climate change progresses, and for geographic neighbor *L. betsileo*. B.) Projected habitat for both species in 2070.

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